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TESTICULAR GRAFTING

from ape to man

Operative Technique
Physiological Manifestations
Histological Evolution
Statistics

Text and Thirty Nine Illustrations

TRANSLATED BY

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WORKS ON GRAFTS AND GRAFTING

BY DOCTOR SERGE VORONOFF

Greffes ovariennes — Comptes rendus du 25e Congrès français, Paris, 1912

Résultats éloignes des Greffes ovariennes — Comptes rendus du 16e Congres International de Medecine, Londres, 1913

Greffe de la glande thyroïde — Academie de Medecine, Paris, 30 juin 1914

Greffe de la peau — Société de Medecine et de Chirurgie, Bordeaux, 30 novembre 1915

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Traité de Greffes humaines — 1 vol Paris, 1916, Octave Doin et fils éditeurs

Greffes osseuses — Comptes rendus du 28e Congres français de Chirurgie, Paris, 1918

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Vivre — Etude des moyens de relever l'energie vitale 1 vol, Paris, 1920 Grasset, éditeur

La Glande génitale mâle et les Glandes endocrines — (En collaboration avec M Retterer) 1 vol, Paris, 1921 Doin, éditeur

Greffes testiculaires — 1 vol Paris 1923 Doin, éditeur

Quarante-trois greffes du singe à l'homme — 1 vol, Paris, 1924 Doin, éditeur

Greffe animale — 1 vol, Paris, 1925 Doin, éditeur

Applications utilitaires de la greffe au Cheptel — Association Francaise pour l'Avancement des Sciences 1926

Etude sur la Vieillesse et le Rajeunissement par la Greffe — 1 vol, Paris, 1926 Doin, éditeur

Etat actuel et l'Avenir des Greffes glandulaires — Universite de Liége, 1927

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ENGLISH EDITIONS

Ovarian Grafts — Comptes rendus XVIIth International Congress of Medecine London 1913

Life (A Study of the Means of Restoring Vital Energy and Prolonging Life) — Published in 1920 by E P Dutton & Company, 181 Fifth Avenue New York

Rejuvenation by Grafting — Published in 1925 by George Allen & Unwin Ltd Ruskin House 40 Museum Street W C 1

The Study of Old Age and my Method of Rejuvenation — Published in 1926 by The Gill Publishing Co Ltd 3 & 5 Paul Bakehouse Court, E C 4

The Conquest of Life — Published in 1928 by Brentano's Ltd, 31 Gower Street W C 1

INTRODUCTION

Since the appearance of our more recent publications, entitled "The Study of Old Age and my Method of Rejuvenation" and "The conquest of life," further facts and information have accumulated. The large number of testicular grafting operations dealing with the implantation of simian grafts upon human tissues, which we have thus far performed permits a general view of the entire question both physiologically and from the standpoint of the histological evolution undergone by the grafts.

This enlarged survey has been rendered possible because of opportunities of removing grafts in certain of our cases at periods of two years three years and four and one half years after the primary grafting procedure. The specimens thus obtained for examination are quite unique in science consisting as they have of testicular simian grafts removed from man after periods never hitherto attained even with autoplastic and homoplastic grafts. Histological sections of these grafts studied by professor Retterer of the Faculty of Medicine of Paris and also examined by one of us have proved the prolonged survival of the glandular cells

present in simian grafts and the perfect correlation existing between physiological phenomena observed after grafting and histological changes in the grafts.

We are also now able to present an exact tabulation of the physiological manifestations observed during these long periods. These manifestations have added precision in that they have been studied not only in animals but also in men, with whom it has been possible to make functional examinations of various systems and organs, including determination of the blood pressure and basal metabolism, ergographic record of muscular energy and similar tests, as well as biological examination of the humors, a procedure which is extremely difficult to undertake with animals. We have also been able to observe in man phenomena of physical, psychical and mental order which are impossible to study save in man.

In addition to these recent data, we are now in a position to present statistics on grafting covering a ten-year period. We are thus enabled to indicate the types of cases in which our method yields the best results and we can also present the proportion in which durable success may be expected in the renewal of grafts after primary grafts have ceased to be active.

However, we desire, before fully entering upon this subject, to emphasize the point that the results which we have obtained, histologically as well as physiologically, have been due to a special and personal grafting method which has nothing in common with methods of

other operators whether published before or after our own communications We must insist somewhat upon this point because, in order to compare the results obtained by various investigators, it is first of all necessary to fulfil the essential requirement defined by Claude Bernard, namely the observance of identical test conditions In order that there may be no confusion upon this point we shall once more give a description of our grafting method elaborated after many years of laboratory study, which has been presented in various publications since the year 1919

This method as applied to a large number of human subjects, has yielded positive results, both physical and mental not only to ourselves but to our faithful collaborators Darligues and Georges Voronoff who have performed hundreds of grafting operations and also to a number of others Among the latter are Baudet, attached to the surgical services of various Paris hospitals Professor Rocher, surgeon of the hospitals of Bordeaux Cochez surgeon of the hospitals at Algiers Le Gatellier, hospital surgeon at Paris Lambret, surgeon at the hospitals of Lille Lalis Bey hospital surgeon at Alexandria Egypt Marro, hospital surgeon at Turin N Pende collaborating with Luigi Durante surgeon at the hospitals of Genoa Pratt hospital surgeon at Nice Schleier, of Vienna Spurr, surgeon at the hospitals of Buenos Aires, Behdjet Sabit, hospital surgeon at Constantinople Tuffier hospital surgeon at Paris Max Thorel, surgeon at the American Hospital

Chicago, Belmiro Valverde, hospital surgeon at Rio de Janeiro, Ferrero Velasco, of Madrid; Madureiro, of Lisbon, Carlos Fortes, of Porto, Portugal, Edwin Creed at Valparaiso, Le Roy des Barres at Hanoï, Francis J M Cathy and J Reeng at San Francisco, G Gibier Rambaud at Paris, and a number of other competent surgeons and investigators

Technique employed by S Voronoff in testicular grafting

In this technique there are certain rules which must be strictly observed concerning the donor animal the operative act and the receiver of the graft

With respect to the donor animal, the operative act itself and the receiving animal these rules cannot be dispensed with As an operative type we will take testicular transplantation from the ape to man which we were the first to recommend

I — Rules concerning the donor

a) *Zoological similarity* It has been clearly established that auto-grafts succeed almost always that homografts succeed often and that hetero grafts succeed but rarely

Auto-grafts employed frequently and with great success with respect to the skin bones cartilage or articulations have almost no application to the endocrine glands the preservation of which is essential to the proper life of every organism Hetero grafts have no practical utility on account of the difficulty of obtaining and applying suitable material All studies concerned with transplantation should especially consider homo grafts which are easily obtained and applied

when the necessary technical conditions are duly observed. With respect to the replacement of an absent gland or the supplementing of a deficient gland in man, the supreme object of our labours, it may be very readily understood that glandular homo-grafts are but very rarely applicable for, save in very rare cases, it is impossible to make use of the organs of one individual for transplantation into the organism of another.

In view of this difficulty, we have selected, as a donor animal possessing the closest zoological similarity with man, the anthropoid ape, which most nearly approaches the human species in the highly perfected development of its tissues and in its intimate humoral chemism. In making grafts of the endocrine glands of these larger apes, which are closely related with man, anatomically and physiologically, the graft employed is really not a hetero-graft, but a graft between two closely related species, such as may be made between the dog and wolf or between the rabbit and the hare.

The graft employed between species so closely related as this is very far from the hetero-graft and on the other hand is closely related to the homo-graft. It may be termed "homeo-graft," the organs of one species finding identical conditions of life in the organism of a nearly allied species.

The age of the ape employed needs ample consideration. Animals which are too young and employed before the establishment of puberty and the production of free hormone secretion are unsuitable. Neither

can animals meet grafting requirements if they are too old and producing too little, or no, hormonic secretion

Being unable to determine the exact age of the larger monkeys we have acquired through long experience a knowledge of certain landmarks or characters permitting us to determine whether the given monkey is adult or young We establish this point by means of the canine teeth, which should be much longer than the incisors and of a light yellow colour When the canines and incisors are at the same level, the fact shows that the animal has not yet attained puberty Teeth of dark or blackish hue constitute a sign of age

Sections of the testicles have confirmed these points The pulp of the young simian testicle has the colour of fresh butter while that of aged apes is dark grayish, or blackish in hue the cells being invaded by pigmentation which is characteristic of old age in these organs Satisfactory health of the monkeys must, naturally, be verified by sufficiently prolonged observation after their arrival from Africa Especially would we emphasize the absolute necessity for blood examination made in order to verify the absence of spirochetosis with which the monkeys are sometimes affected in the absence of any signs of the infection We never make use of any animal which has not been previously examined at the Pasteur Institute and our example should be followed by all who practise grafting of this kind

Operation upon the monkey for extraction of the

testicles from the scrotum, presents nothing of special interest. It may be remarked, however, that the scrotum of the monkey is small and that the testicles lie very close to the root of the penis. The important point consists of the manner of treating the testicle of the monkey for grafting purposes, for upon it success or failure of the graft may depend.

When liberated from its envelopes the testicle should remain attached to its vascular pedicle until grafting is completed, in order that the interruption of the life of the grafts during their transfer from one organism to the other may be reduced to a minimal period not exceeding one or two minutes.

The histological examination of a gland detached from its vascular connections and preserved for some hours or days in a suitable solution or in cold storage, reveals nothing special in its cellular structure. Glands so preserved may be usefully employed for opotherapeutic purposes or for the preparation of powders or extracts but they are unsuitable for grafting.

The cells possess something other than their histological aspect. They possess *life* invisible microscopically. It is the life of the cells which must be preserved during the transfer from one organism to another. The diminished biological activity of a gland which is momentarily detached from its connections may be intensified by sufficient blood supply but the latter can never restore dead cells to life. Experience has taught us that resumption of the life of the gland when it is graft-

ed and therefore the maintenance of biological continuity, require practically instantaneous transfer from the donor to the receiver

On removal from its recess the testicle is first separated from the epididymis in order to leave only the gland for grafting The gland is then divided into fragments which are supplied as they are required, while remaining constantly irrigated with blood derived from the vascular pedicle The size of the fragments is of great importance An experience of nearly twenty years has proved that it is not practicable to treat all endocrine glands in the same way

In the process of grafting the behaviour of the cells of different glands differs according to the gland employed The suprarenal gland for example when transplanted either in its entirety or by smaller or larger fragments becomes absorbed with surprising rapidity in a few days only Two years devoted to experiments upon a large number of animals have shown us that the extremely differentiated and finely developed cells of this gland cannot preserve their vitality during the delay of some hours required for the formation of a new vascular supply The suprarenals can be grafted only by anastomosing their vessels directly, by the Carrel method

The thyroid on the contrary may be readily grafted either by implanting large fragments or bits no larger than the head of a pin by using the technique of Kristiani The latter procedure is inapplicable to the ova

ries or testicles. Small fragments of these glands become resorbed very rapidly, yet the other extreme is equally unacceptable. Unduly large fragments of the testicle undergo necrosis because they are not irrigated throughout their entire thickness by the newly formed vessels which are produced with our technique, but which possess only a limited capacity for penetrating tissue. For these reasons, therefore, fragments used for grafting must be neither too small nor too large. Histological examinations of the grafts at various stages of their development have shown that, in order to permit the new blood supply derived from the host to reach all of the transplanted glandular substance, the thickness of the graft should not exceed one centimeter.

When employing the relatively small testicle of the chimpanzee or the cynocephalous ape, it suffices to divide the testicle into four equal parts by incising in two directions, first splitting the testicle at its middle and then separating each half into two parts. However, fragments so obtained are still too thick, and are wedge-shaped, like slices of melon (Figs. 1 and 2) (1). The sharp edge should be trimmed flat with the scissors, to leave a thickness of about one centimeter (Fig. 3). Histological sections show that some of the newly formed vessels penetrate the graft through the albuginea. It is therefore well to scarify the albuginea

1 The nine figures showing the various stages of the graft have been taken from the work *The Renewal of the Organism*, by our friend and collaborator, Dartigues.

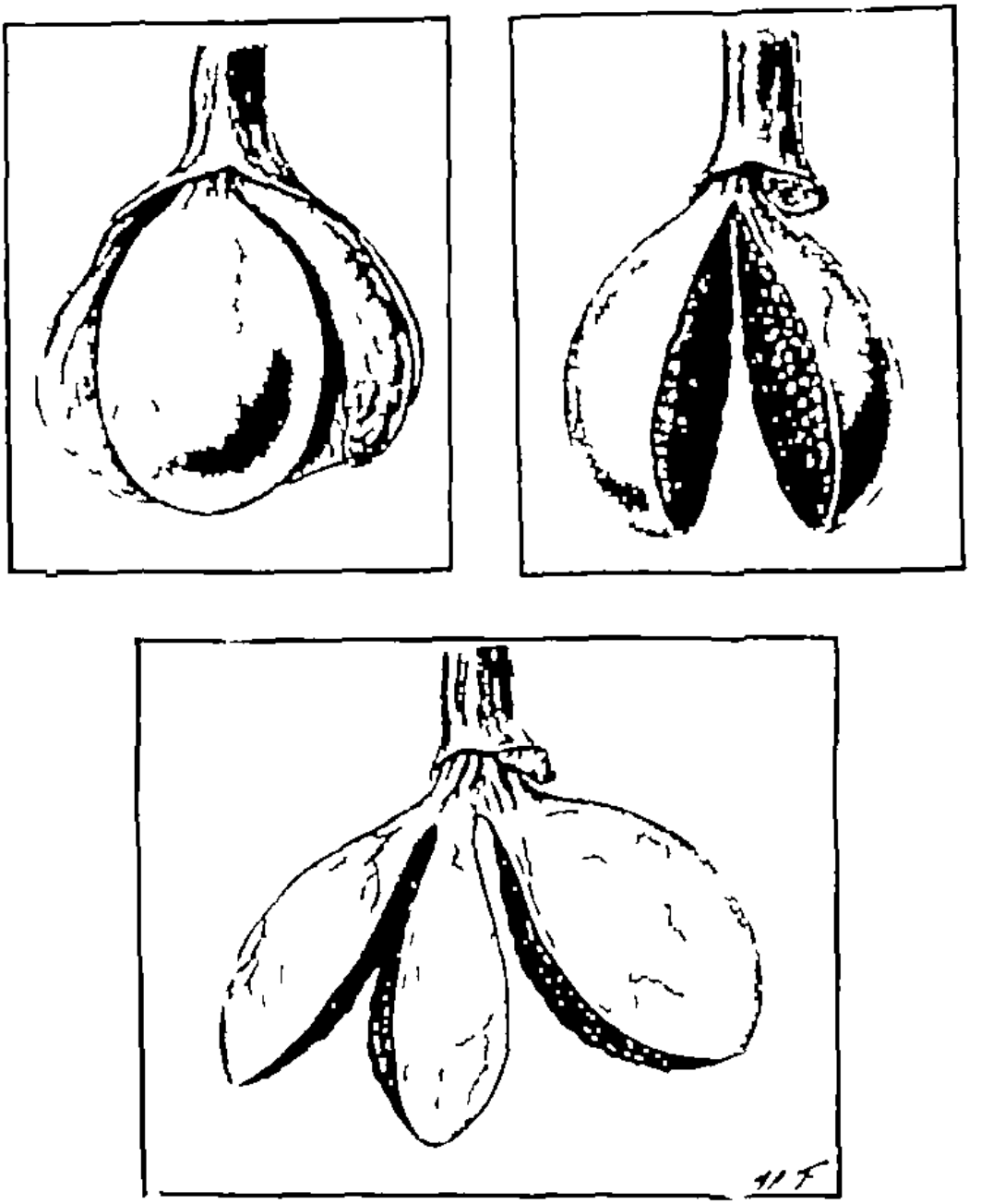

FIG. 1 — OPERATION ON THE SIMIAN DONOR

Division of the simian testicle into four grafts

Above the figure at the left shows the testicle attached to the spermatic cord the tunica vaginalis resected and the epididymis detached

The figure at the right shows the testicular ovoid suspended by the spermatic cord with complete resection of the tunica vaginalis and the valueless epididymis and divided into two equal parts

Below the figure shows the right half still entire of the simian testicle the left half being divided to form two grafts

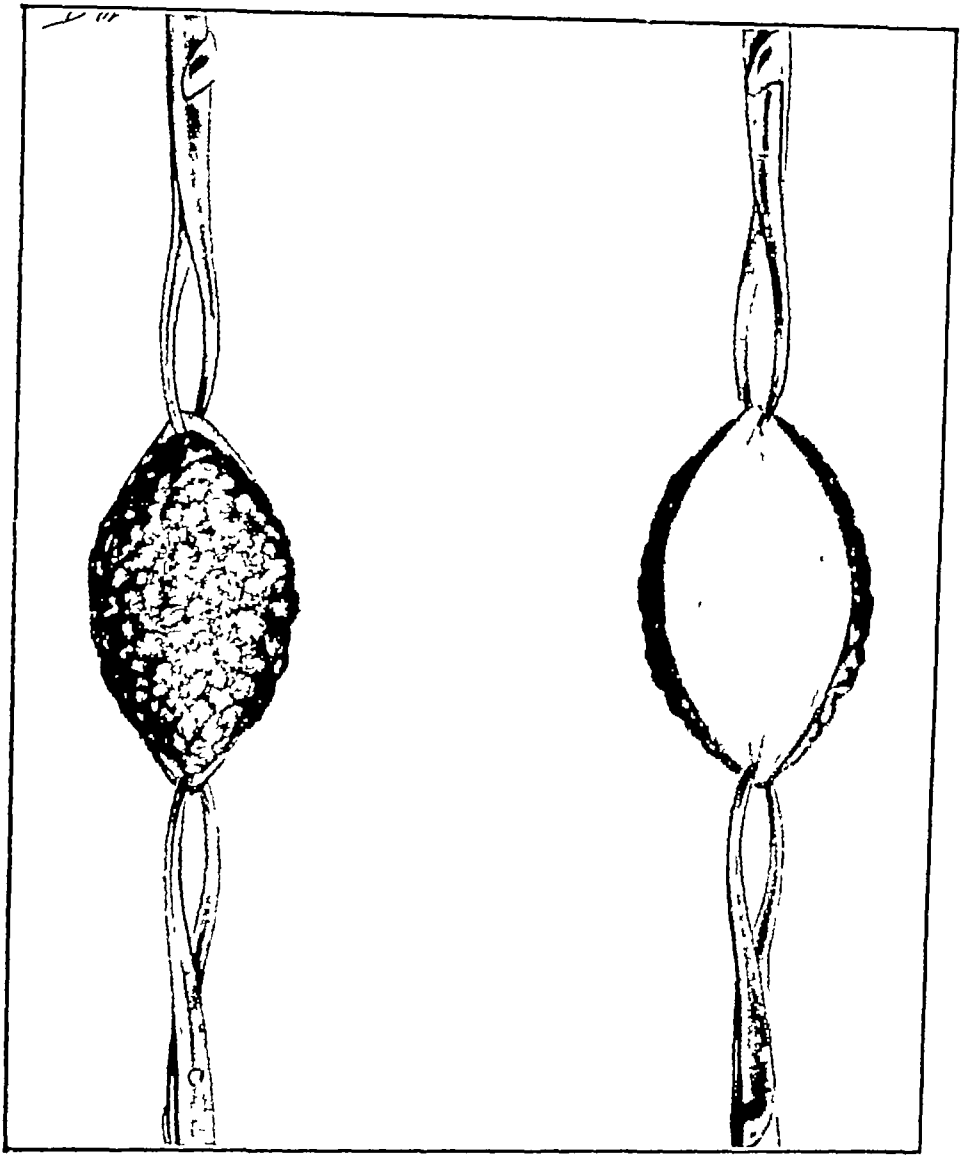

Fig. 2

OPERATION ON THE SIMIAN DONOR

Making the simian graft.

On the left the detached graft, viewed from its albugineal aspect. On the right, the detached graft, viewed from its pulpar side, which is to be applied to the parietal layer of the tunica vaginalis.

Fig 3

OPERATION ON THE SIMIAN DONOR

Shaping the simian graft

The testicular pulp is often redundant Some of it may be removed with the curved scissors to reduce the thickness of the graft to about one centimeter

invariably selected, namely, the scrotum, for the implantation of our testicular grafts.

II. Rules concerning the receiver

a) *Fixation of the graft* — There is thus no doubt that the graft should be made within the scrotum, but just what site should be selected? For determining this point, it has been necessary, first of all, to ascertain how a graft, or, in other words, the survival of a transplanted gland, may be secured

The studies by Carrel and our own experimental work, dating from 1910, have shown us that the life of a transplanted gland is intimately connected with its blood supply As our purpose was not the removal of existing organs and their replacement by organs taken from another organism, we could not resort to the direct anastomosis of vessels. At all events, this is impracticable because of the small calibre of the spermatic vessels supplying the testicles For obtaining true grafts under the unfavourable conditions present, it was necessary to find a way of producing the formation of new vessels, for nourishing a new organ We therefore conceived an idea which we shall discuss further on.

For the moment, let us examine the anatomical structure of the scrotum, in order to find within it a site which is favourable for the possible creation of new and numerous vessels. For this purpose, it is evidently

necessary to seek a tissue as rich as possible in blood vessels from which may arise a new network of capillaries The dartos, the cellulo-erythroid membrane and the albuginea contain very few blood vessels The tunica vaginalis, on the other hand and especially the external surface of its parietal layer are provided with a very rich vascular supply In fact, this membrane may be identified by the numerous vessels which ramify in it and cover it completely Save for this well irrigated site there remains only the testicular pulp, or the glandular structure itself, as a region provided with sufficient blood supply

However our long experience in general surgery has taught us the risks accompanying incision of the testicle consisting of hemorrhage and the consequent production of cicatricial tracts which are injurious to testicular vitality Our first aim should evidently be "primum non nocere " Before relying upon new testicles, it were prudent not to injure those already existing By attempting implantation within the testicle itself only insignificant fragments could be introduced without absurdly evacuating more or less of the existing glandular pulp

The only site available within the scrotum for the implantation of the grafts is evidently the tunica vaginalis and especially the external surface of its parietal layer Here then the grafts must be attached The internal surface of the parietal layer can be employed for this purpose only in rare cases as when hydrocele

the survival of a new gland taken from another organism It continues to live and perform its function in the organism to which it has been transferred and to secrete its hormone regularly during a certain number of years We have based this principal operative act, which is the determining factor in grafting, upon the following principles

Living matter reacts in different ways, according to the conditions in which it is placed. For example, a modification in the normal rhythm and functioning of a given organ, produced by congestion, alters the aspect and action of the organ Owing, thus, to congestion or inflammation, of any origin whatever, the flow of blood to the organ is increased, the local arteries become engorged, the blood can no longer be contained within the vessels and first infiltrates the neighbouring tissues and then forms new channels In this way new capillaries at first occur, somewhat larger vessels are next formed and an entire new network is finally produced Renal congestion constitutes an excellent example of this process

Under special conditions, then, new vessels may be formed and we need only find ways of producing these conditions artificially at the site selected for grafting The method which we found applicable proved to be very simple, consisting only of producing irritation of the surface of the tunica vaginalis by scarifying it with the tip of the knife or by scratching it with a needle (Fig 6). In this way congestion, which is, naturally,

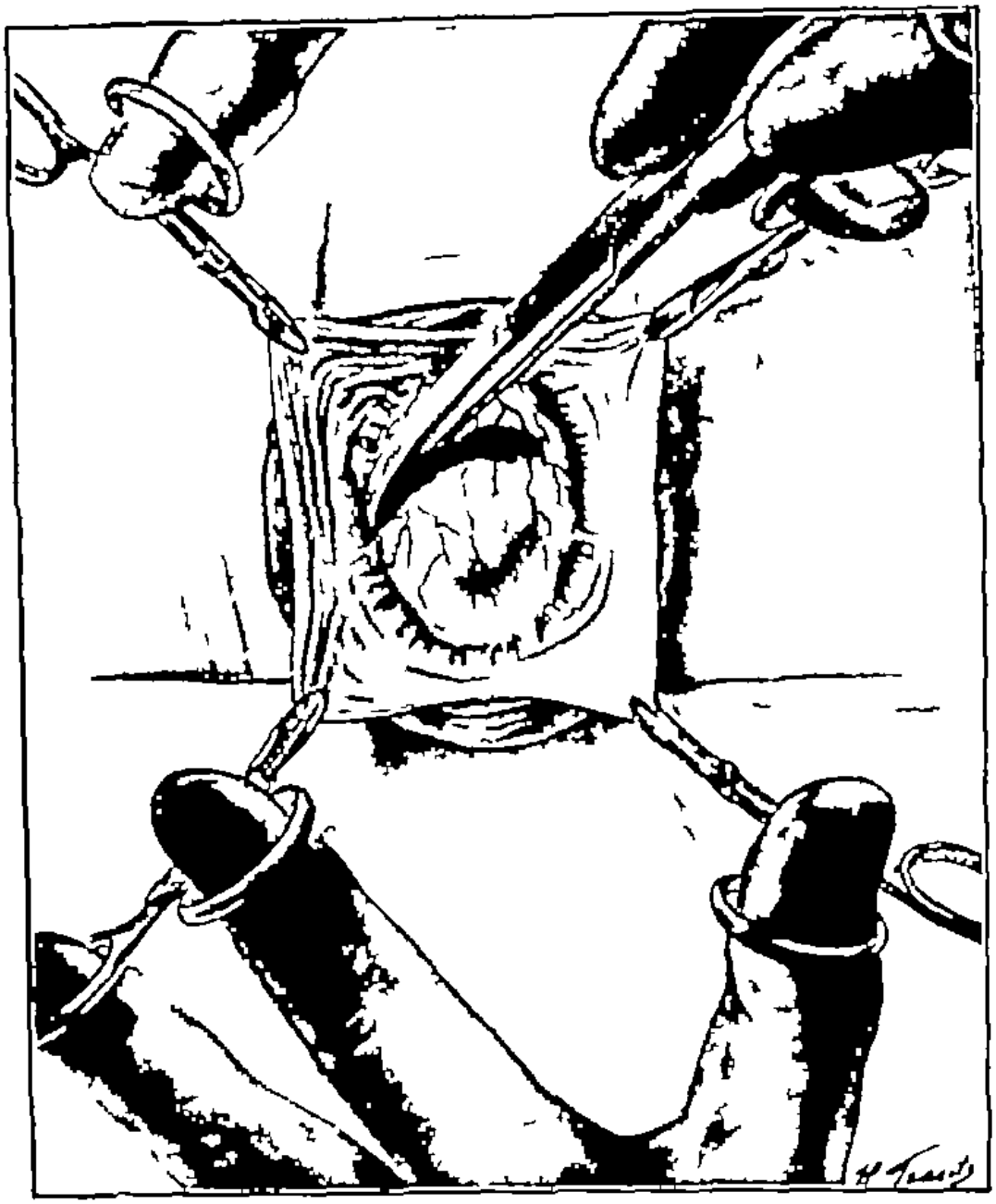

Fig 5

OPERATION ON THE HUMAN RECEIVER

Preparation for fixation of the grafts and isolation of the parietal layer of the tunica vaginalis

The cellulo fibrous membrane being divided vertically the tunica vaginalis attached by cellular tracts is loosened with curved scissors The grafts will be placed in each lateral sinus produced by the detachment

aseptic, is brought about and continues for several days, new blood vessels being formed They may often be encountered in the glandular substance of the grafts as early as the fourth day following transplantation, while the vessels originally present in the grafts often resume their function of conveying blood within a very few days after operation. Conditions permitting survival of the grafts being thus assured, it remained to find the best circumstances for fully utilizing them.

With this end in view, it was first necessary to establish the relation between the quantity of blood required for maintaining the life of the cells of the graft and the capacity of the vaginal surface to supply this quantity This relation could be established only by experimenting. After many trials, we learned that the thickness of each graft should not exceed one centimeter and that not more than two grafts should be implanted upon each tunica vaginalis. If the grafts be more numerous than this, their vascular supply is insufficient, they undergo necrosis and are resorbed as are organic foreign bodies, having no viable connections with the receiving organism The two grafts in question, trimmed as described above, must be placed with their glandular surfaces closely in contact with the external surface of the tunica vaginalis, which has been suitably prepared to receive them They must lie well apart, not in contact

The surface of the tunica, which is to give rise to the newly formed vessels, must be quite free about the

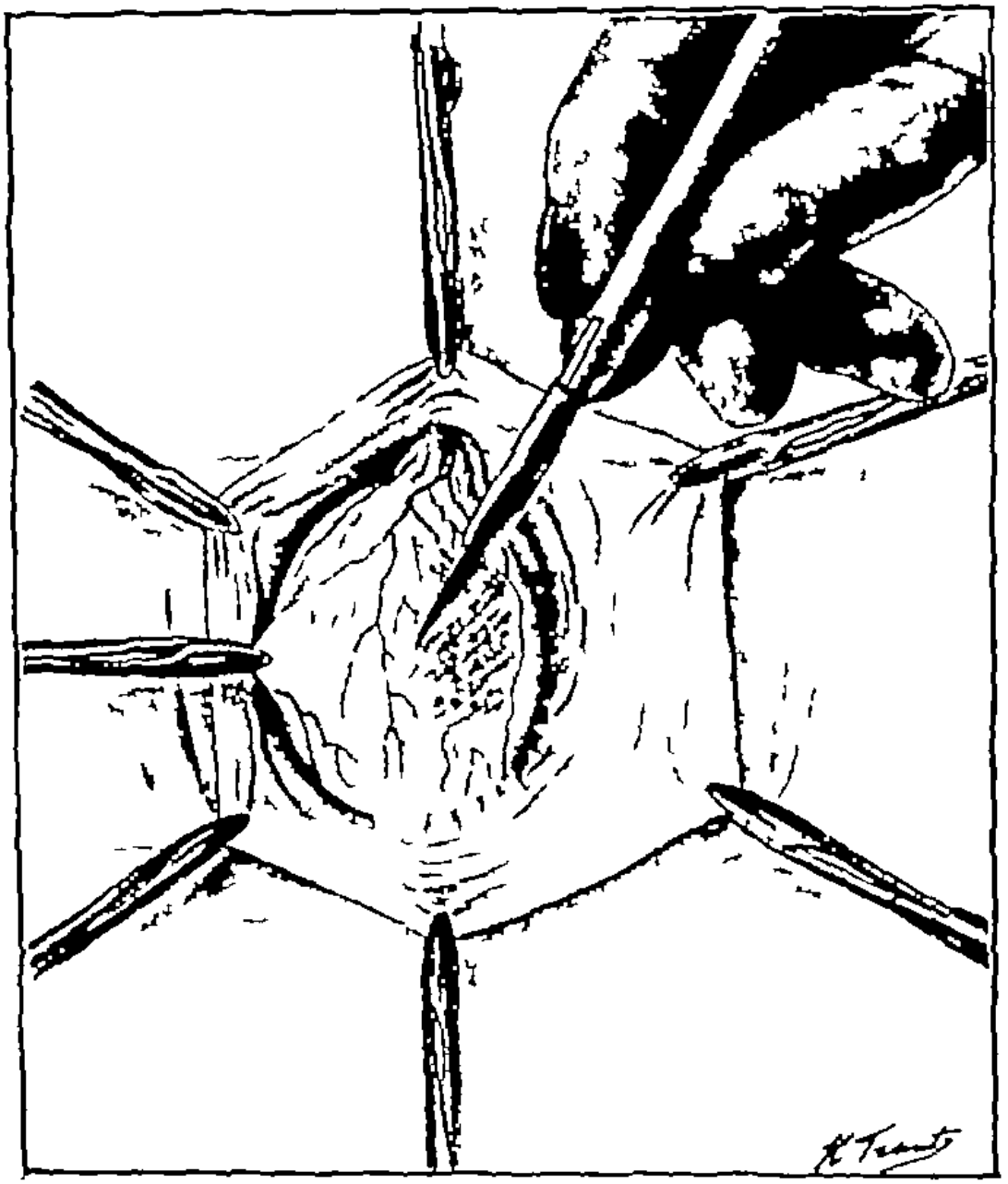

Fig. 6

OPERATION ON THE HUMAN RECEIVER

Freshening the grafting surface by scarification

This is the most important and critical point of the operation Here scarification is being performed with the tip of the knife The point of a Reverdin needle may also be used

Physiological phenomena observed after grafting

Having fully described our technique, we shall now present the physiological phenomena which we have observed after grafting operations in which this technique has been employed

In the majority of cases marked psychical and sexual excitation occurs in man during the first few days following testicular transplantation The patients believe that this abrupt phenomenon is a sign of the beneficial effects of the procedure However, these results continue only for a few days and the supposed good effects rapidly diminish and usually completely disappear For the first two months and often during the first three months after grafting the patient experiences no beneficial effect whatever, at least so far as objective evidence is concerned This period is one of disappointment disillusion and discouragement, and commonly follows the enthusiasm present during the immediately earlier period

However, the satisfaction and pleasure of the patient become so much the greater later on In the course of two or three months marked improvement in the psychic functions occurs especially in physicians and other individuals who are highly developed intellectually

There are improvements in memory, greater aptitude for mental labour and greater facility for intellectual effort. At the same time, the genital functions become more active and a general condition and sensation of well-being occur. Such are the earlier phenomena observed

More concrete evidence soon appears. The physiognomy undergoes a change, the eyes become brighter, the skin is firmer, more elastic and of better colour, some of the wrinkles disappear and the face expresses a certain euphoria. A remarkable effect noted by many observers is increased activity of the pilar system, especially in parts where the hairy growth is an evidence of masculine virility, such as the beard, the front of the chest, the abdominal linea alba and the pubic region. The hair grows freely, is of glossy appearance and its colour is sometimes that originally present The effects upon the hair are due to the graft, and solely to the graft, since the hair of regions governed by the thyroid secretion, such as the eyebrows, eyelashes and hair of the scalp, is not affected Modifications and similar changes affecting the hairy growth occur also in sheep, in which the wool becomes more abundant and of better quality after grafting.

The general attitude and appearance of the grafted individual become more youthful, the enfeebled body becomes more vigorous, tonus and muscular force increase (as shown both subjectively and objectively, by dynamometry) and movements are more supple and sure. The subcutaneous fat largely diminishes The

digestive functions reflect the improvement, the appetite increasing, while functional gastric dilatation, gastro-intestinal flatulence and atonic constipation diminish and even wholly disappear owing to increased tonicity of the smooth muscle tissue

With respect to the circulatory system, the most surprising effect is the constantly observed lowering of blood pressure in individuals affected with hypertension Explanation of the mechanism of this result is difficult Possibly it may be due to increased tonicity of the small vessels or of the structures regulating blood pressure, or, again, the newly introduced testicular hormone may, perhaps, have an inhibitive action upon the adrenal secretion the morphological and physiological increase of which occurs regularly in old age

Improvement in urinary evacuation so frequently disturbed in old age by vesical atony and accompanying prostatic hypertrophy is also produced As a result, dysuria and pollakiuria diminish and even disappear This phenomenon may be partly assigned to increased tonicity in the muscular fibres of the bladder and partly to vicarious diminution in prostatic congestion Many pathologists consider prostatic congestion and hypertrophy as compensative phenomena, from the hormonal viewpoint, produced in consequence of diminution in the endocrine secretion of the testicle (Kenneth M Walker)

Improvement in the organs of the special senses sometimes occurs especially in the eye and

AN EXAMPLE OF THE EFFECT OF THE GRAFTING PROCEDURE (1)

Before operation

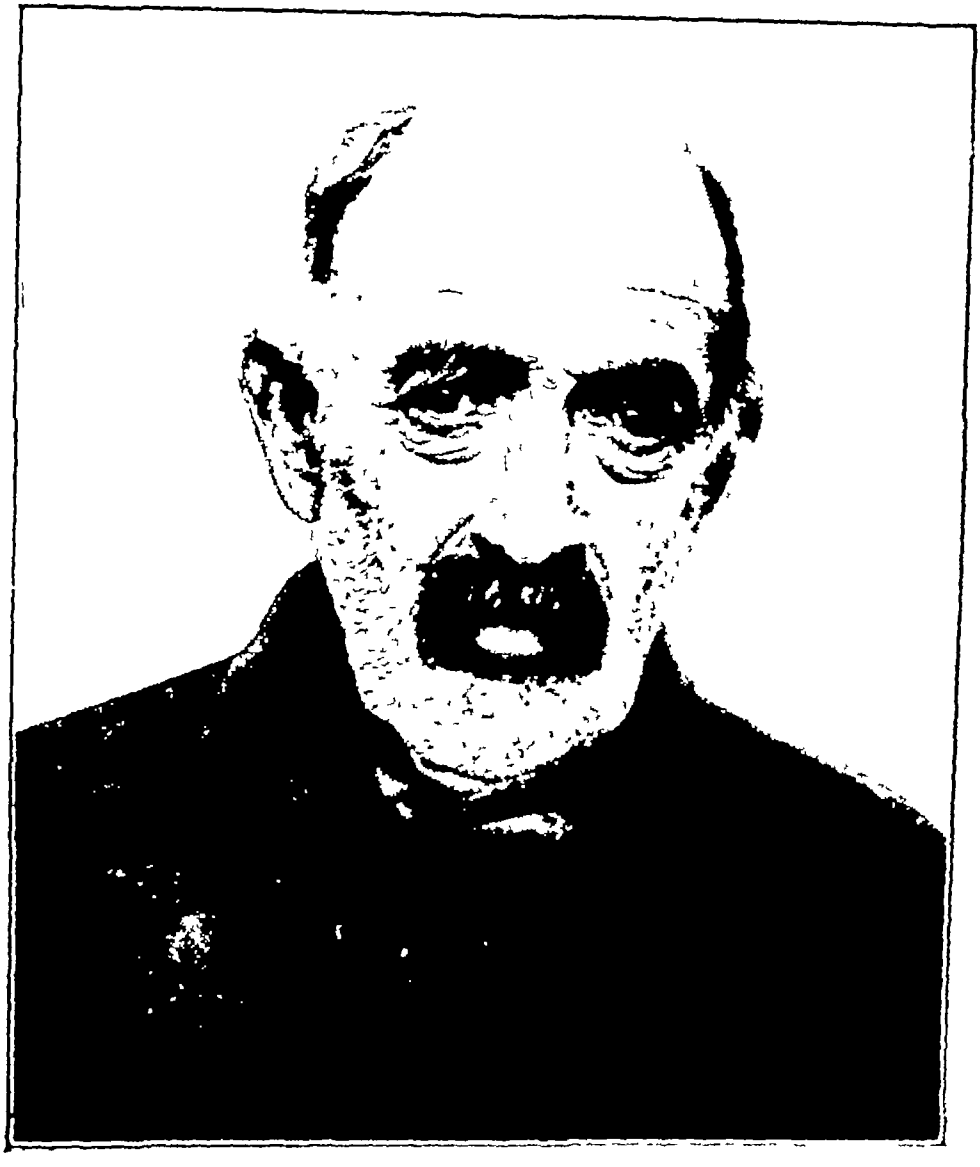

Fig. 10

Mr. Georges Behr,
of the Old Men's Home at Douera, near Algiers, at the age of seventy-three years

AN EXAMPLE OF THE EFFECT OF THE GRAFTING PROCEDURE

After operation

Fig 11

Mr Georges Behr, at the age of seventy-six years

Photograph taken at the civil hospital at Algiers

cases of presbyopia This favourable effect is probably due to increased tonicity of the ciliary muscles.

Finally, functional and biological tests show the occurrence of favourable changes in the general metabolism Cholesterin and urea become diminished, the basal metabolism rises and glycemia and glycosuria decline in cases of diabetes accompanied by hypertension

A general survey of the results indicates that the favourable phenomena described above, appearing a few months after operation, persist from three to five years After this time, the morphologic and dynamogenic effects produced by the graft begin to diminish, and completely disappear after the fifth or sixth year

Let us now examine the structure of the grafts during their life within the organism to which they have been transplanted and let us also learn whether their evolution does not present phases which are capable of explaining the series of physiological phenomena following the grafting procedure (1).

1 The homoplastic grafts have been made in part by Voronoff and in part by Didry, Voronoff's preparator at the Collège de France

A descriptive study of testicular grafts removed at intervals ranging from a few hours to four and one-half years after transplantation

Before presenting the findings derived from the examination of histological sections of simian grafts removed from the human host two three and four and one half years after transplantation, we believe that it will be useful to study sections of grafts removed from different animals at intervals ranging from a few hours to two years The evolution of the grafts, thus presented histologically in an unbroken series ranging from twenty four hours to four and one half years, will thus appear precisely in a manner showing all the evolutional phases which the grafts undergo from the moment of their implantation

It is quite superfluous to give a detailed description of the hundreds of sections which Retterer and one of us have made since 1917 Our study will therefore deal only with features representing the most characteristic phases of the histological evolution

1. Homoplastic graft, Dog N° 162. Graft removed after 24 hours (November 17, 1928).

A. Macroscopic description. — Graft, 12 mm. long, 6 mm wide and 9 mm. thick. Of reddish-yellow colour and firm and elastic consistency.

B — Technique — Fixed in Zenker-Helly solution and formol-ammonium bromide Sections 1 to 3 micra thick taken from pieces imbedded in paraffin, and 5 micra thick taken from frozen sections Stained with hematoxylin-eosin-orange, Van Gieson Mallory and Sudan III

C Microscopic description — a) *Topographic examination* Microscope Zeiss, objective 16 mm double ocular Mobili N° 15

The graft retains a practically normal structure and its anatomical independence, there being no tissular connection between it and the tissue of the host (Figs 12 and 13) The few special details present are the following the fibrous offshoots of the albuginea are slightly distended and separated, the collagen fibrils being swollen and partly rendered individual. Between these fibrous plates, the nuclei of fibroblasts, surrounded by a fine oxyphilic border of cytoplasm are clearer and more regular. The seminiferous tubules, round or oval in cross-section present the usual diameter of 150 to 200 micra Their membrana propria is intact and their content consists of cells of full vitality which fill the tubules almost

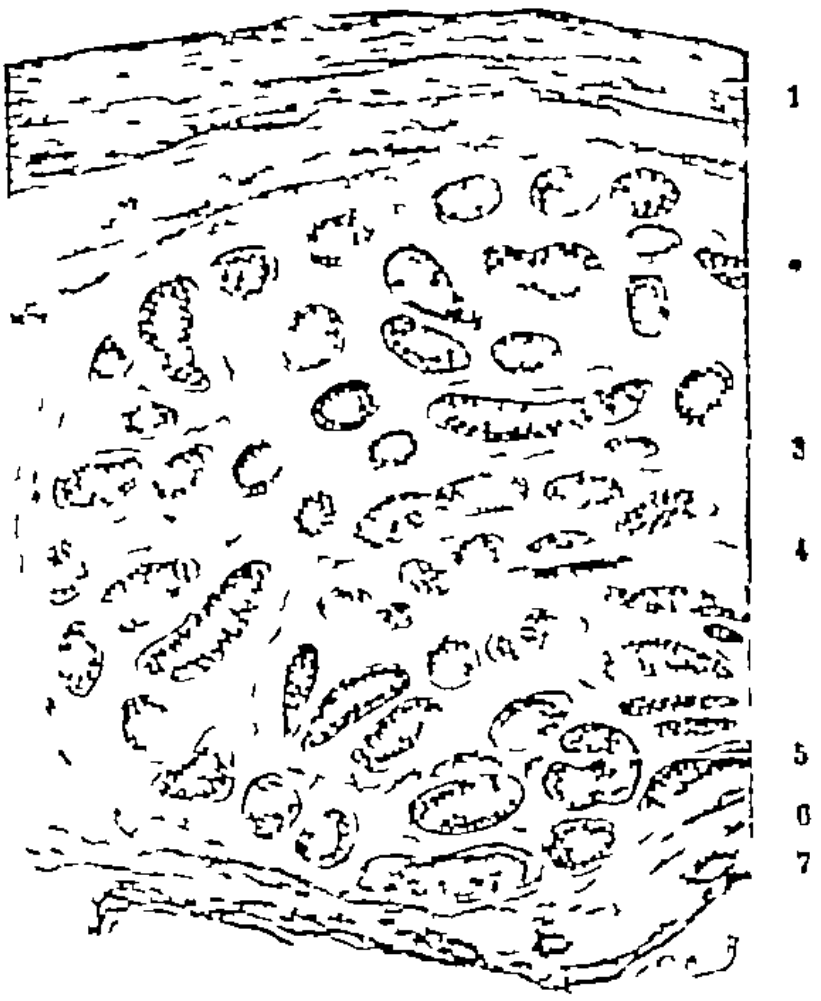

FIG 12

Testicular graft removed after 24 hours

(*Enlargement 40 diameters*)

1 The albuginea of the graft — 2 Seminiferous tubules — 3 Space produced by infiltration of plasma — 4 Interseminiferous connective tissue — 5 Cellular infiltration — 6 Connective tissue from the host — Dilated vessel perivascular infiltration

completely. A characteristic fact is the separation of the tubules, which are normally tangent to each other, with very little interspace hardly more than two or three micra across, while the tubules of the sections lie 40 to 60 micra apart. The intertubular spaces are but partially occupied by loose connective tissue, most of the spaces being empty or partly subdivided by fibrous filaments. The appearance suggests that the separation of the tubules was produced mechanically by infiltration with liquid, of which traces remain after the dehydrating process of the histological technique.

At the periphery of the graft, in the region of its attachment, the intertubular spaces contain numerous polynuclears, plasmocytes and red cells, showing an evident tendency to infiltrate the tissue centripetally. The interseminiferous connective tissue is represented by widely separated fibres, by adult connective-tissue cells and by a very few of the interstitial cells of Leydig. Throughout the entire section, scarcely more than two or three of these cells may be identified, and those not very surely.

The host tissue shows nothing particular save a dilatation of the vessels, most of which are surrounded by numerous elements which have migrated from the blood. There are strings and nodules of polynuclear cells and a few plasma cells scattered among the connective-tissue fibres, especially toward the region upon which the graft is attached.

b) *Cytologic examination.* Microscope, Zeiss im-

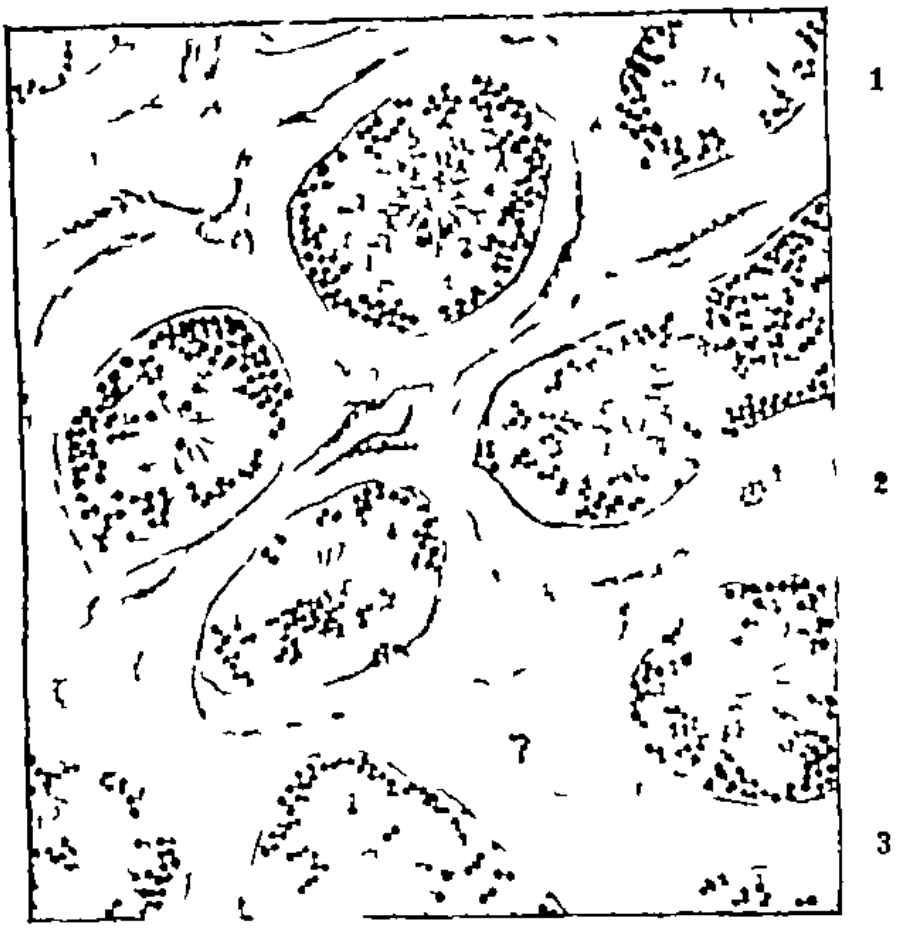

Fig 13

Testicular graft removed after 24 hours

(Enlargement 180 diameters)

1 Seminiferous tubule — 2 Interstitial connective tissue — 3 Space produced by infiltration of plasma

mersion objective, double Mobili ocular N° 15, enlargement 2 000 diameters

The structure of the seminiferous tubules is almost normal (Fig. 14) The walls are almost intact. At certain points, their structure is discontinuous, owing to abnormal distension and separation of the fibres All generations of the seminal cells occur within the tubules, the spermatids being especially abundant and almost wholly filling the tubular lumen The numerous spermatozoa lie mostly in the central portion, but many of them have infiltrated into other regions, where they lie amid other seminal cells The cells of Sertoli present nothing abnormal, except that the usual appearance of the "candelabra", due to collections of the spermatozoa about the thinned-out portion of the cells, is absent The cytoplasm of the spermatogonia and the Sertoli cells contains numerous vacuoles and fuchsinophilic granules. The method of Regaud shows that the same cells also contain mitochondrial granulations grouped about the nucleus, especially in the infra-nuclear region, the part of the cell lying nearest to the membrana propria being considered the base

Rod-like, crystalline formations occur in some of the cells, especially the Sertoli cells Frozen sections stained with Sudan and scharlach show numerous spherules of neutral fat. In the interstitial tissue, the fibro-connective-tissue elements show nothing special Even minute examination reveals only two or three clearly characteristic cells of Leydig

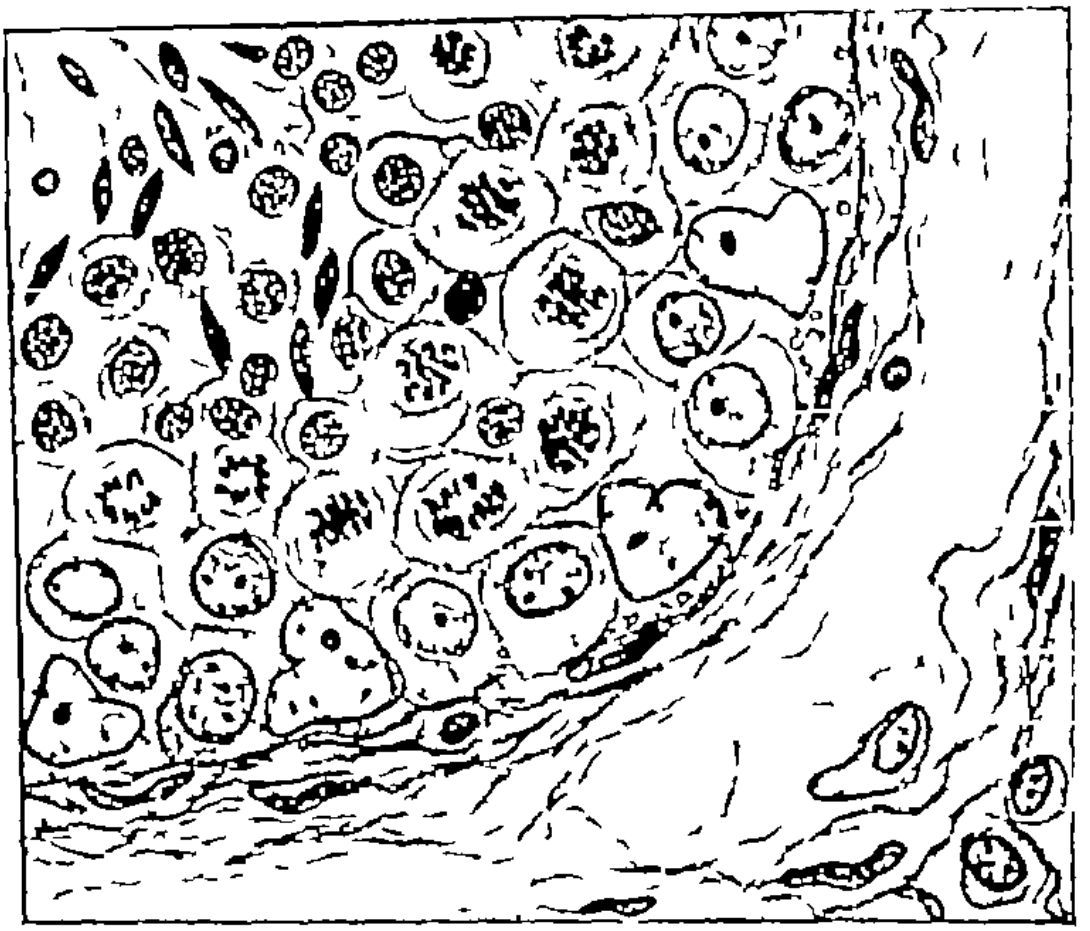

Fig 14

Testicular graft removed after 24 hours

(Immersion objective enlargement 1 200 diameters)

Appearance of a seminiferous tubule and of the interstitial tissue

1 Membrana propria of the seminiferous tubule layers separated — 2 Sertoli cell — 3 Spermatogonia — 4 Spermatocyte — 5 Spermatid — 6 Spermatozoon — 7 Interstitial connective tissue (fibres) — 8 Epithelioid cell (Leydig?) — 9 Plasma cell. — 10 Connective tissue cell — 11 Space produced by infiltration with plasma

Between the peripheral tubules occur very numerous cells derived by infiltration from the host tissue Most of them are neutrophilic polynuclears, the plasma cells, lymphocytes and red cells occurring more rarely

Summary A graft removed after 24 hours presents the following characters the seminiferous tubules are filled with cells of normal structure The intertubular spaces are wider, the increased separation of the tubules being due partly to liquid infiltration (probably plasmatic) and partly to cellular infiltration at the periphery of the graft The interstitial tissue shows no sign of alteration or proliferation

2. Homoplastic graft, Dog N° 169. Removed after 4 days (November 22, 1928).

A Macroscopic description — Graft, 8 mm long, 5 mm wide and 4 mm thick Colour reddish white firm and elastic consistency

B Technique — Fixation with Zenker-Helly and formol-ammonium bromide Sections of 1 to 3 micra from pieces imbedded in paraffin and stained with hematoxylin-eosin-orange, Van Giesen, Mallory and Regaud Frozen sections of 5 micra, stained with Sudan III and scharlach

C Microscopical description — a) *Topographic examination* — Microscope, Zeiss, objective 16 mm , double Mobili ocular N° 15

The graft retains many characters permitting its recognition, but many special features exist, of which the more important follow (Figs 15 and 16) The albuginea of the graft is infiltrated by numerous polynuclears and plasmatic cells , its fibres are swollen The dimensions of the seminiferous tubules are still almost normal, the diameters ranging from 160 to 200 micra The staining of the peripheral tubules indicates that their vitality is wholly normal Those toward the central portion of the graft present unquestionable vitality in the first cellular layers, but the layers lying more centrally show autolysis as indicated by homogeneous and diffuse staining pyknosis and fragmentation of the nuclei Distances between the tubules range from 40 to 60 micra, and the connective tissue is infiltrated by numerous lympho-connective tissue cells both at the periphery and the centre of the graft

At the point of attachment tissular adhesion is beginning between the graft and the host tissue Many collagen fibrils and fibroblasts may be followed from one tissue to the other A few fine, newly formed capillaries occur occasionally at this site Some of the original vessels of the graft are filled with red cells and leucocytes, in good condition together with transplanted hematic cells showing signs of autolysis

b) *Cytologic examination* — Microscope, Zeiss, 1/12 immersion objective, double Mobili ocular N 15, enlargement 2 000 diameters

The structure of the peripheral seminiferous tubules

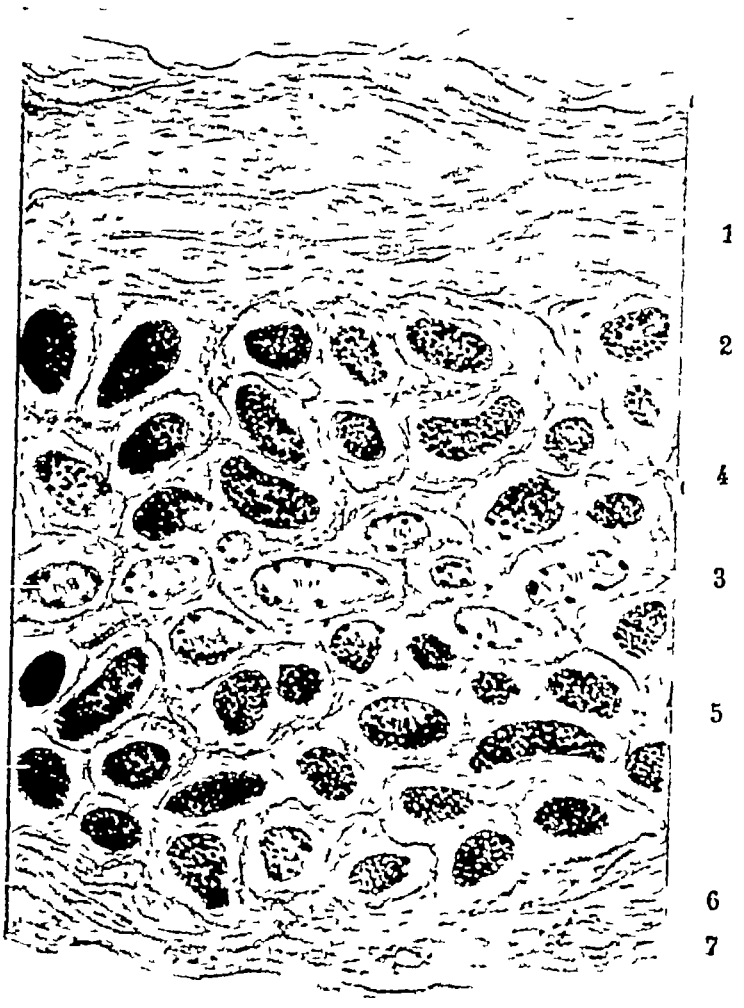

FIG 15

Testicular graft removed after 4 days.

(Enlargement 40 diameters)

1 The albuginea, disintegrated and infiltrated — 2 Peripheral seminiferous tubule, filled with living cells — 3 Central seminiferous tubule, necrobiotic — 4 Young connective tissue — 5 Cellular infiltration — 6 Connective tissue from the host — 7 Dilated vessel, perivascular infiltration

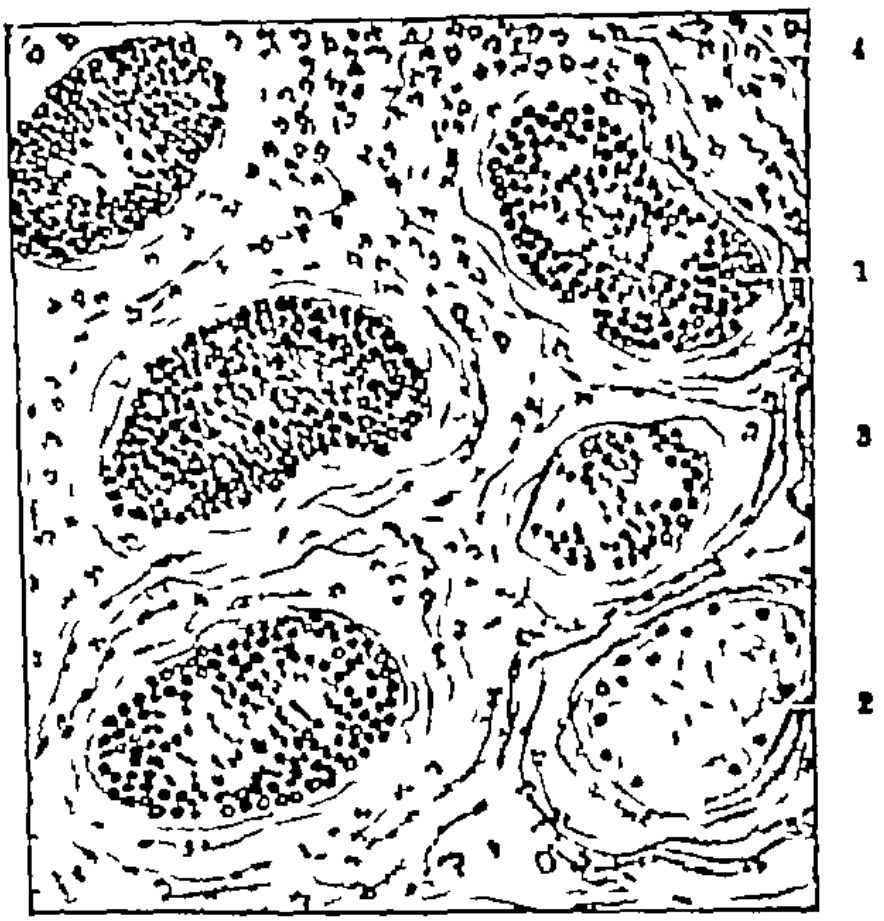

Fig 16

Testicular graft removed after 4 days

(*Enlargement 180 diameters*)

1 Peripheral seminiferous tubule filled with living cells. — 2 Central necrobiotic seminiferous tubule. Sertoli cells still present. — 3 Interstitial connective tissue — 4 Leucocytic infiltration

is almost normal and, in many ways, comparable with that observed after 24 hours, the well-filled circles and columns containing all generations of seminal cells, together with the rows of Sertoli cells (Fig 17). Most of the walls present evident discontinuity, the lamellar fibres being separated and ruptured at different sites Cells from the tubules are mingled with the lamellae and, at some points, they can be traced beyond this limit, amid the cells of the interseminiferous or interstitial connective tissue. A few polynuclears have also infiltrated into the distended tubular wall

Tubules at the central part of the graft retain the Sertoli cells and columns of the spermatogonia, and their centre is occupied by spermatozoa. Here the spermatids and spermatocytes present cytolysis, condensation, nuclear fragmentation and homogenization of the cytoplasm. Both in the peripheral and central tubules, the first cellular layer has a clearly glandular structure, with abundant protoplasm, numerous vacuoles, fuchsinophilic granulations, mitochondrial granules and crystalline formations.

The interseminiferous tissue is very rich in young fibroblasts. The elastic and connective-tissue fibres are relatively few, but the collagen fibrils are very numerous. Lying between the connective tissue elements there occur in places, especially near the tubules, epithelial cells which are isolated or form small groups, with large amblyochromatic nucleus and distinct nucleolus, and surrounded by abundant cytoplasm containing

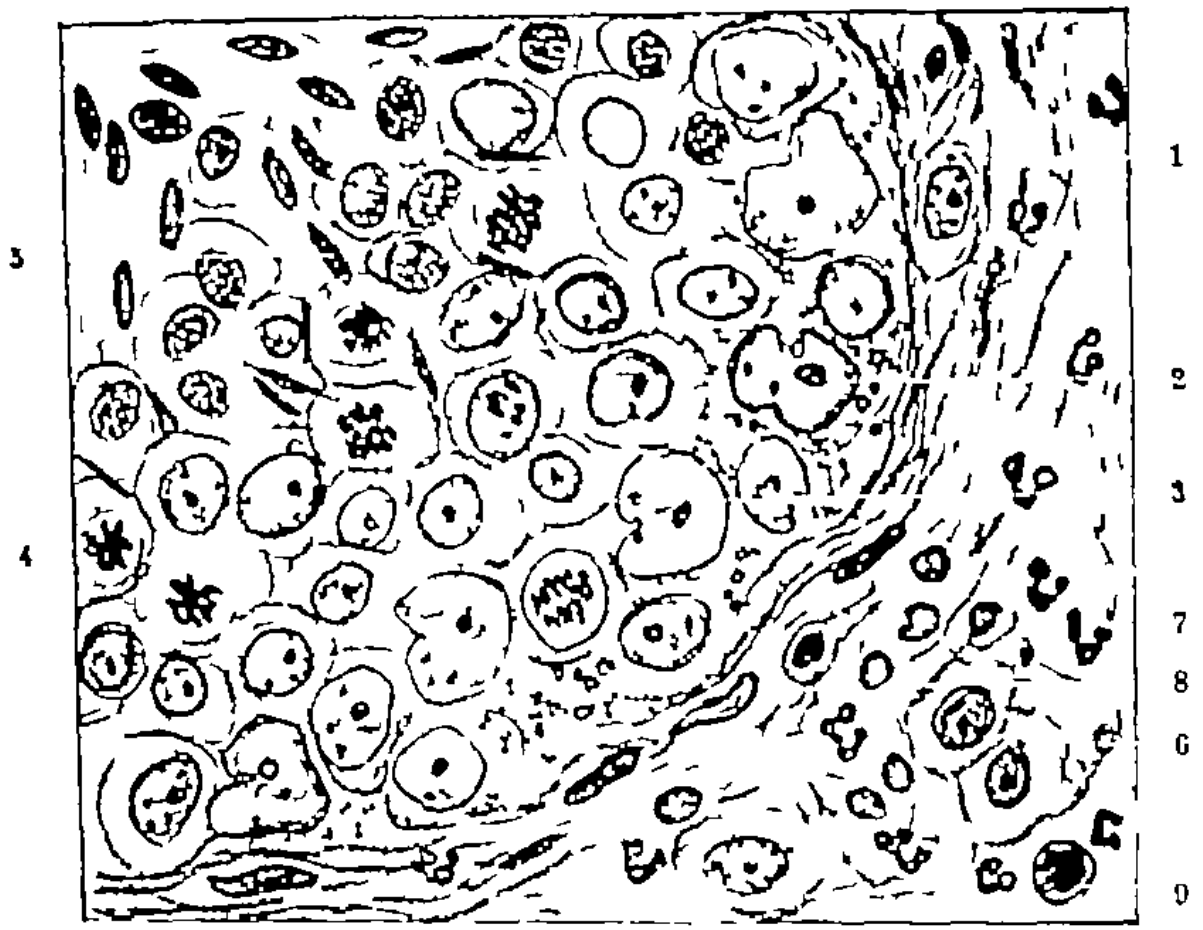

Fig 17

Testicular graft removed after 4 days

(*Enlargement* 1 200 *diameters*)

1 Membrane of a seminiferous tubule layers infiltrated and widely separated — 2 Sertoli cell — 3 Spermatogonia — 4 Spermatocyte — 5 Spermatozoon — 6 Interstitial (Leydig) cells — 7 Polynuclear leucocyte — 8 Red cell — 9 Plasma cell

many granulations and crystalline rods Up to a certain point these cells may be identified as ordinary Leydig cells, but it must be noted that the tubular cells have characters and structure very similar to those of these cells. Outside these cells, the intertubular mesenchymatous elements are infiltrated from every direction, in the peripheral region, as well as centrally, with numerous polynuclears, lymphocytes, plasma cells and red cells The small vessels noted at the topographic examination, penetrating to a depth of about 1 mm , are limited to an endothelial wall, covered with a very fine perithelial reticulum Their diameters range from 8 to 12 micra At the same point of attachment, young connective-tissue cells are also penetrating and a rich collagen reticulum has formed and is filled with hematic cells derived from the host

Summary Four days after implantation, the graft has the following appearance and structure the tissue of the graft is more or less penetrated from the tissue of the host by connective tissue and vessels, which tend to unite the two structures The peripheral seminiferous tubules, transformed ino full columns by the proliferation of the seminal cells, retain complete vitality Tubules at the central part of the graft, whose first layers of cells (spermatogonia and Sertoli cells) remain alive and intact, present signs of autolysis in the spermatocytes and spermatids The spermatozoa are situated centrally or distributed irregularly, and are of normal aspect.

Hyperplasia of the connective tissue predominates in the interseminiferous tissue which is infiltrated with numerous hematic cells and lympho-connective tissue elements The isolated and grouped cells, of glandular aspect and similar to the Leydig cells are increased At the point of attachment, there are a few newly formed capillaries and some of the original vessels in deeper parts of the graft have been refilled with blood

3 Homoplastic graft, Dog N° 149 Removed after 7 days (November 30, 1928)

A Macroscopic description — Graft, 8 mm long, 5 mm wide and 4 mm thick Colour reddish white consistency firm and elastic

B Technique — Fixation with Zenker Helly and formol ammonium bromide Sections of 1 to 3 micra from pieces imbedded in paraffin stained with hematoxylin eosin-orange Van Gieson, Mallory, Regaud Frozen sections of 5 micra stained with Sudan III and scharlach

C Microscopic description — a) *Topographic examination* — Microscope Zeiss objective 16 mm , double Mobili ocular N 15

In the graft two zones are evident on first view A peripheral portion belonging partly to the graft and partly to the point of the host s attachment, has the staining and morphological characters which denote

vitality. A central portion is of homogeneous aspect after basic staining and, while showing evidences of a testicular section, permits no particular structure to be differentiated. This region is nearly normal (Figs. 18 and 19).

In the peripheral zone, the tubules have diminished in size, their diameters ranging from 100 to 140 micra They are generally round in outline, but the latter is not clearly defined. Their content consists of living cells, of little variety. They almost wholly fill the tubules, yet leave a small central lumen. As already indicated, their walls are no longer continuous and the transition from tubules to intertubular spaces is difficult to perceive at certain points Between these tubules of the peripheral zone lies connective tissue, very rich in its own elements (young and old connective-tissue cells) and infiltrated cells, most of which are hematic

Very fine blood vessels are also present in this interstitial tissue They are newly formed, of 10 to 12 micra in diameter and limited to endothelium and perithelium Original vessels of the graft are also distinguishable, and are full of hemoglobinic and leucocytic cells of the blood.

In the central zone, the seminal tubules are narrowed, uniformly stained, and contain cellular structures which are very difficult to distinguish It is a remarkable fact that some tubules contain, centrally, a few well-stained heads of spermatozoa At certain points, the intertubular tissue has the characters denoting a certain

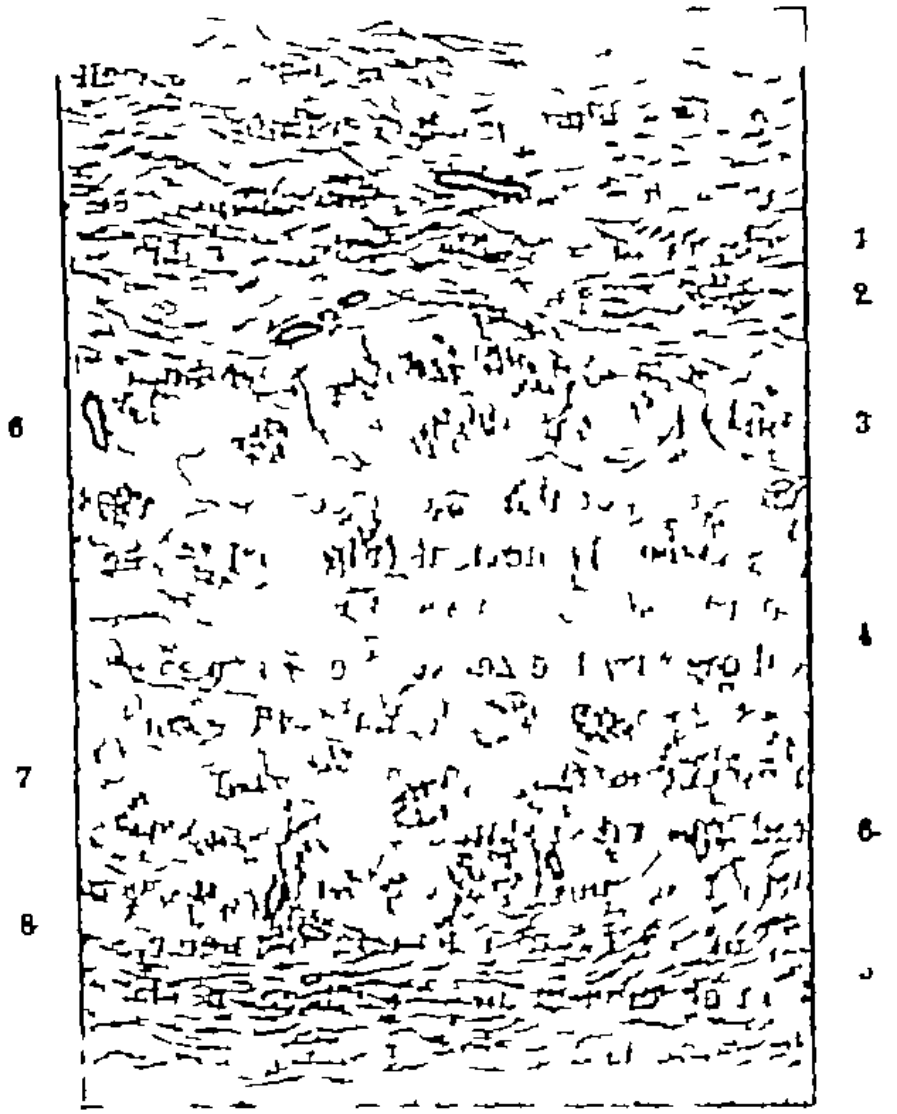

Fig 18

Testicular graft removed after 7 days

(Enlargement 60 diameters.)

1 The albuginea of the graft — 2 Dilated vessel perivascular infiltration — 3 Peripheral seminiferous tubule filled with living cells — 4 Central seminiferous tubule necrosed — 5 Connective tissue from the host — 6 Newly formed vessel penetrating the graft — 7 Young interseminiferous tissue — 8 Vasculo-connective tissue zone of attachment.

degree of vitality, but most of this tissue, as well as the tubular tissue, is evidently degenerating

b) *Cytologic examination* — Microscope, Zeiss, 1/12 immersion objective, double Mobili ocular N° 15, enlargement 2 000 diameters

We examine separately the living peripheral portion and the degenerated central zone.

In the peripheral portion, the structure of the seminiferous tubules is nearly normal (Fig 20). The Sertoli cells show increased number and size, and are full of vacuoles, as shown by the Zenker-Helly fixation, paraffin imbedding and staining with hematoxylin-eosin-orange Numerous mitochondrial granulations are brought out by the Regaud method of staining Cells of seminal type present a certain morphologic uniformity, most of them being round, of 15 to 20 micra in diameter, with a nucleus rich in chromatin and an evident nucleolus, the surrounding cytoplasm being frankly oxyphilic and relatively rich in osmiophilic and fuchsinophilic granulations.

Karyokinetic figures are relatively infrequent, but spermatozoa are fairly numerous, either at the centre of the tubules or infiltrated together with other cellular elements As mentioned in the topographic examination, the membrana propria of the tubules no longer forms a continuous base, nor is it well defined The connective tissue lamellae are much detached, widely separated and scattered about in part The cells of the

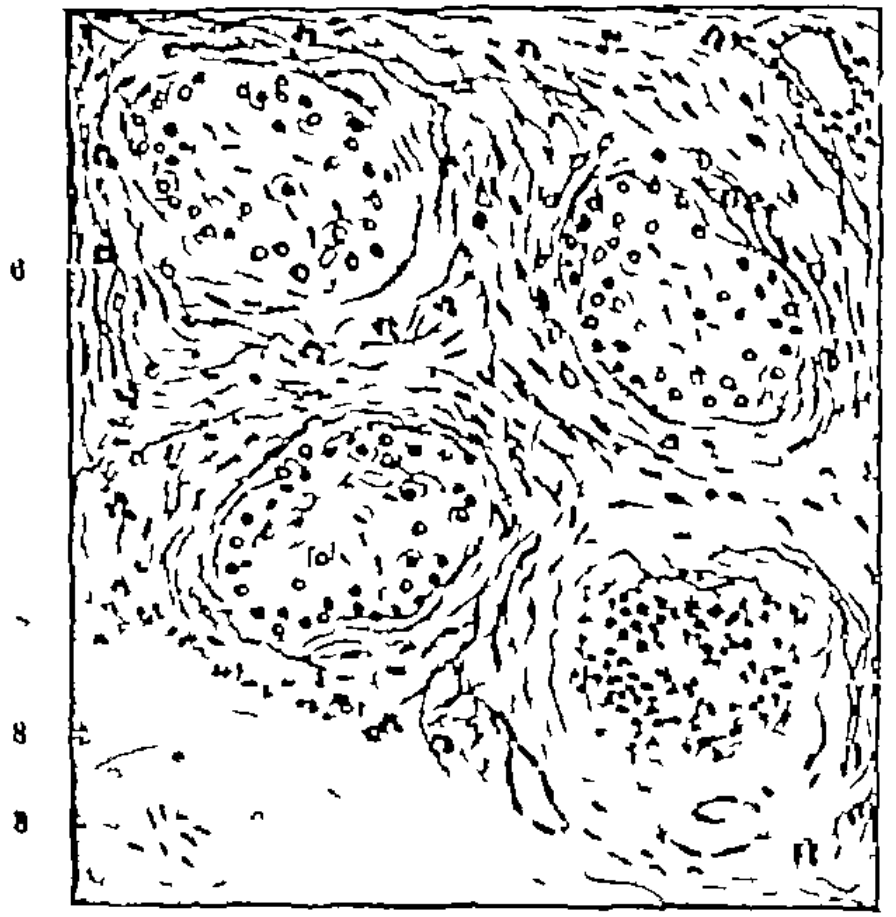

Fig 19

Testicular graft removed after 7 days

(*Enlargement 180 diameters*)

1 Seminiferous tubule containing living cells (beginning of an insular formation) — 2 *Seminiferous tubule being transformed to a pseudo follicle* — 3 Central seminiferous tubule necrobiotic (persistent spermatozoa) — 4 Interseminiferous connective tissue — 5 Newly formed vessel — 6 Infiltrated cell — Leucocytic barrier toward the necrosed zone — 8 Necrobiotic connective tissue

degree of vitality, but most of this tissue, as well as the tubular tissue, is evidently degenerating

b) *Cytologic examination.* — Microscope, Zeiss, 1/12 immersion objective, double Mobili ocular N° 15, enlargement 2 000 diameters

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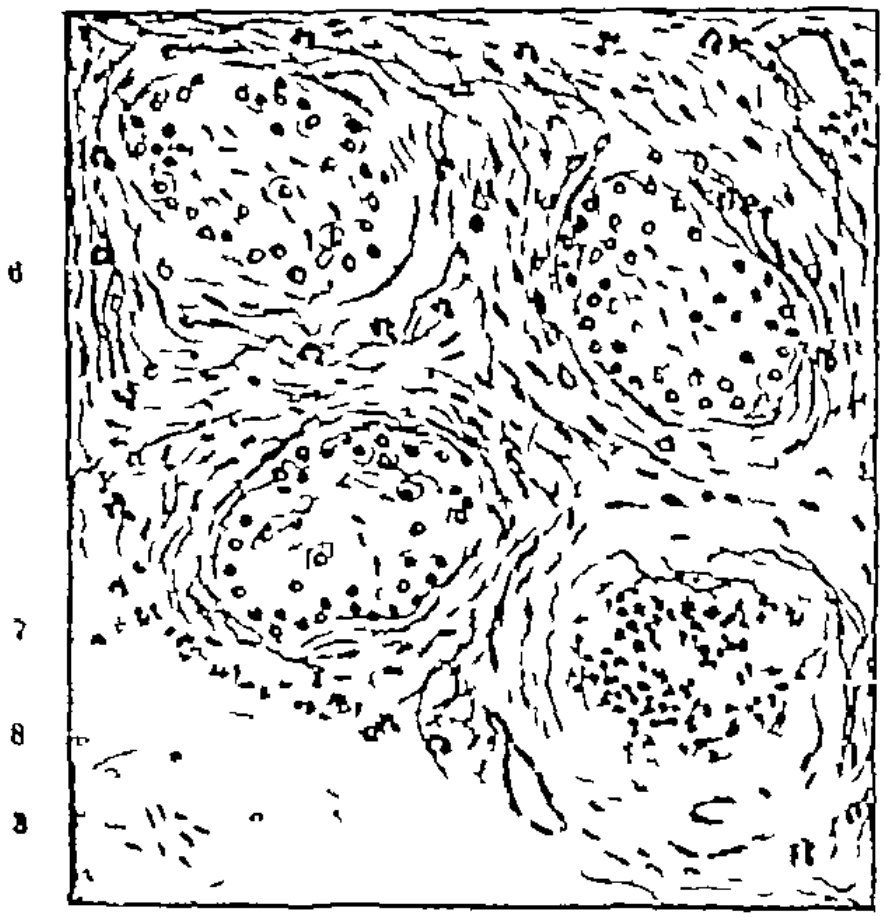

Fig 10

Testicular graft removed after 7 days

(Enlargement 180 diameters)

1 Seminiferous tubule containing living cells (beginning of an insular formation) — 2 Seminiferous tubule being transformed to a pseudo-follicle — 3 Central seminiferous tubule necrobiotic (persistent spermatozoa) — 4 Interseminiferous connective tissue — 5 Newly formed vessel — 6 Infiltrated cell — 7 Leucocytic barrier toward the necrosed zone — 8 Necrobiotic connective tissue

tubules penetrate the interseminiferous tissue and are more or less mingled with it In turn, this tissue is formed of numerous and richly interlaced collagen fibrils, reinforced with bands of old connective tissue fibres, infiltrated as in the preceding case with many young fibroblasts and old connective-tissue cells Leydig cells, or cells of similar type, are present in a rather large proportion Infiltrated and lympho-connective tissue cells are less numerous and many of them present signs of degeneration, such as pyknosis, nuclear fragmentation and homogenization of the cytoplasm The newly formed capillaries and vessels of small calibre have their usual structure.

In the deeper, or central, part of the graft, high magnification confirms the topographic findings. Most of the tubules of the region, together with the interseminiferous tissue, present signs of autolysis and, especially signs of hyaline and granulo-fatty degeneration Two points merit special notice Spermatozoa and Sertoli cells persist in many of the tubules, whose other epithelial elements are wholly degenerated , and the intertubular connective tissue is better preserved and resists autolysis better than does the epithelial tissue

Summary Seven days after transplantation, the graft has the following structure . the peripheral portion is alive and already attached to the host tissue, from which it is penetrated by numerous young connective tissue cells and blood capillaries. The epithelial factor is represented by seminiferous tubules, which are dimin-

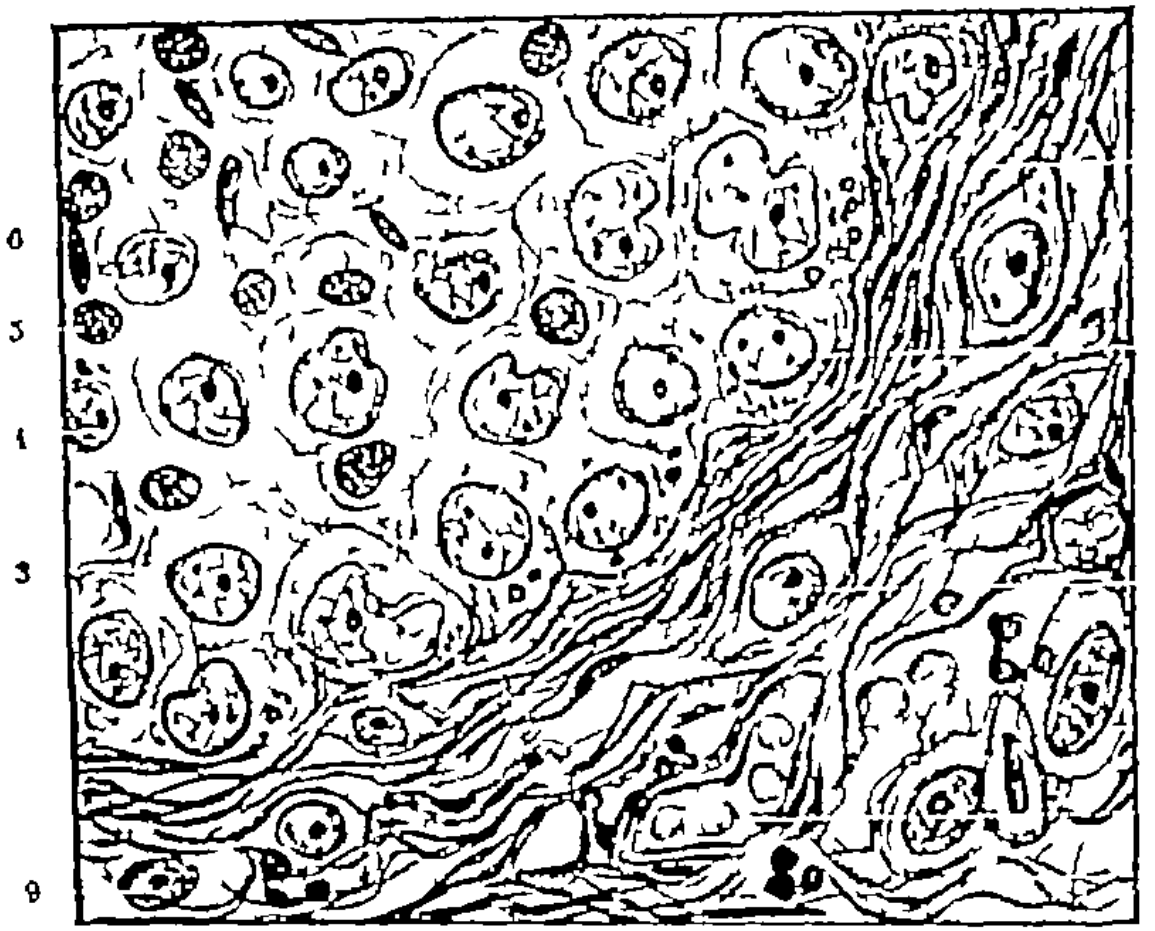

Fig 20

Testicular graft removed after 7 days

(Immersion objective enlargement 1 200 diameters)

1 Membrane of a seminiferous tubule (layers separated and mingled with the interseminiferous tissue) — 2 Abundant Sertoli cells — 3 Abundant spermatogonia — 4 Spermatocyte — 5 Spermatid — 6 Spermatozoon — 7 Epithelioid cell — 8 Interstitial Leydig cell — 9 Plasma cell — 10 Young interseminiferous connective tissue — 11 Newly formed vessel

ished in size and full of cells tending to have uniform morphology The connective tissue is highly hyperplastic and rich in young elements , it contains capillaries and small epithelioid cells

The central part of the graft is necro-biotic, in spite of the persistence of intact spermatozoa and Sertoli cells in some of the seminiferous tubules

4. Homoplastic graft, Dog N° 134. Removed after two weeks (December, 1928).

A Macroscopic description — Graft, 6 mm long., 4 mm wide and 4 mm thick Colour, yellowish white, consistency firm and elastic

B Technique — Fixation with Zenker-Helly, formol-ammonium bromide and Flemming Sections of 1 to 3 micra from pieces imbedded in paraffin Staining with hematoxylin-eosin-orange, Van Gieson, Mallory and Regaud Frozen sections of 5 micra, stained with Sudan III and Nile blue

C Macroscopic description — a) *Topographic examination* — Microscope, Zeiss, 16 mm objective, double Mobili ocular N° 15

There is no longer any clear demarcation between the graft tissue and the host tissue Anatomical union is practically complete (Figs 21 and 22) A preliminary view shows peripheral tissue possessing the characters of

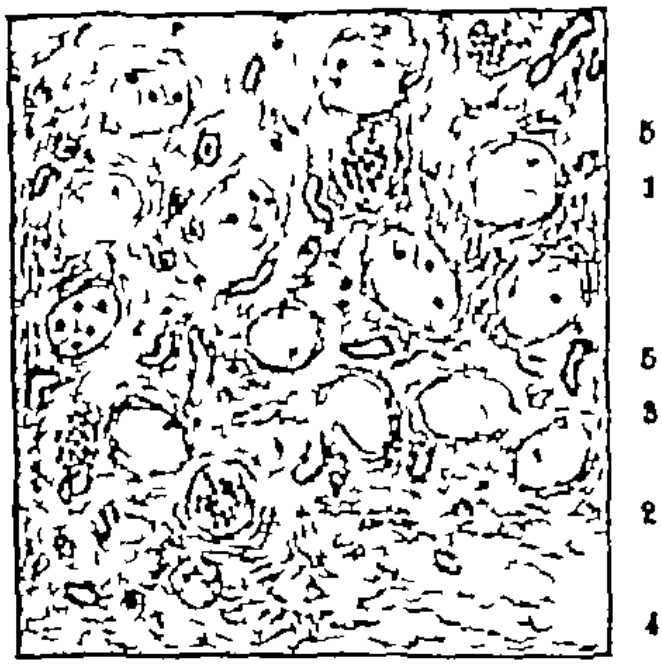

Fig. 21

Testicular graft removed after 2 weeks

(*Enlargement 60 diameters*)

1 Seminiferous tubule transformed into an insular epithelial structure — 2 Seminiferous tubule transformed into a pseudo-follicle — 3 Interseminiferous connective tissue peripheral zone — 4 Interseminiferous connective tissue central zone — 5 Newly formed vessel

vitality and a small central nub of doubtful vitality We shall deal only with the living, peripheral portion

At a depth of 2 to 2,5 mm , the seminiferous tubules are preserved, retaining the characters previously described. The basal membrane is no longer distinct, the content of the tubules consists of cells completely filling the lumen, the dimensions of the tubules have greatly diminished and their diameters range from 80 to 120 micra The distance between them ranges from 30 to 50 micra. The interseminiferous tissue, especially characterized by the youth of its elements, has been infiltrated by a small number of lympho-hematic elements and is penetrated by numerous vessels, of which most are capillaries

b) *Cytologic examination* — The tubules are represented by cells whose characteristic is morphologic homogeneity. Cells of seminal function are mostly represented by round and polyhedric elements, 15 to 20 micra in diameter, arranged in three or four layers Their ambly-chromatic nuclei present granules of chromatin connected by a fine reticulum and the nucleolus is evident. The cytoplasm is clearly oxyphilic and numerous enclosures give it a spongy aspect. In sections fixed with Flemming solution, many of these cells contain osmiophilic granules After staining with Sudan, the cells in frozen sections show large scarlet granulations consisting of neutral fats

The Regaud method shows numerous mitochondria in the first cellular layers. Numerous spermatozoa exist at the

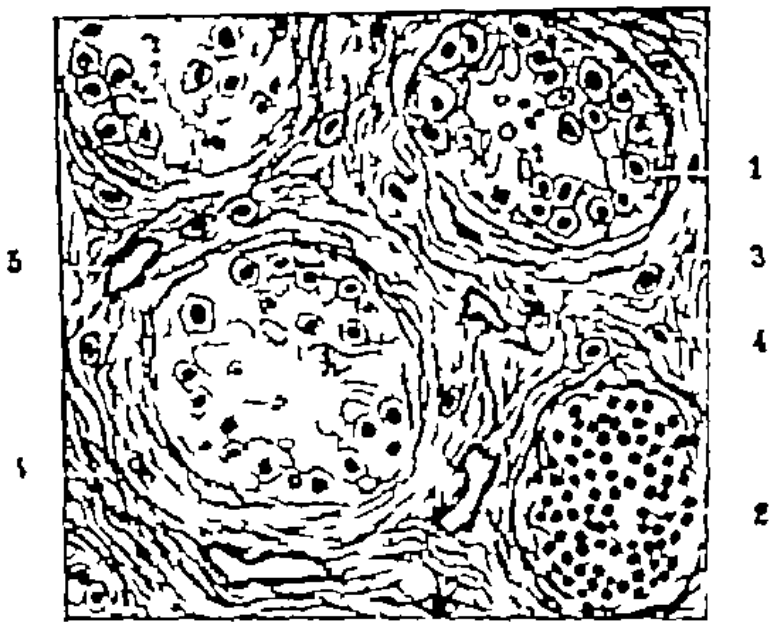

Fig 22

Testicular graft removed after 2 weeks

(Enlargement 180 diameters)

Seminiferous tubule being transformed into an island of uniform prismatic cells — 2 Seminiferous tubule transformed into a pseudo follicle — 3 Interseminiferous connective tissue — 4 Interstitial Leydig cell — 5 Newly formed vessel

centres of the tubules and mixed with the cells which have been previously described. The Sertoli cells can be distinguished from other cells only with difficulty. The histological characters of the tubules in this condition are as follows.

The intratubular cells have marked morphologic homogeneity and karyokineses are but few. The basal membrane is not distinct : the separated connective tissue lamellae mingle without demarcation from the connective tissue fibres of the tissue. The cellular elements of the tubules appear to wander among these fibres, while the cells of the interseminiferous tissue appear at the same time to penetrate the tubules. In the interstitial connective tissue the epithelial cells are abundant and resemble the Leydig cells. The infiltrated plasmohematic elements are less numerous and most of them show fragmentation of the nuclei and cytoplasm, which is surcharged with fat

In the central zone mentioned in the topographical examination, there are lesions characteristic of necrobiosis, but here, as in the other case, the striking fact consists of the presence of the heads of spermatozoa, possessing normal staining reactions, in the central part of the tubules, in which the other elements are degenerate

Summary Two weeks afte ' ntation, the gr
structure shows peripheral s is tubules w
have preserved their character y and whic
formed of full columns Th lar cells h

tendency toward morphological homogeneity The basal membrane tends to disappear and dual penetration is occurring between the cells of the tubules and those of the interseminiferous tissue The interstitial tissue is richly represented by young connective tissue, and presents numerous glandular epithelial cells similar to the Leydig cells The circulation appears assured partly by *means of newly formed capillaries and small vessels* and partly by the resumption of function in original vessels present

5 Homoplastic graft, Dog N° 167 Removed after two months (January, 1929)

A Macroscopic description — Graft 5 mm long 4 mm wide and 4 mm thick Colour yellowish white, consistency firm and elastic

B Technique — Fixation with Zenker Helly formol ammonium bromide and Flemming Sections of 1 to 3 micra from pieces imbedded in paraffin and stained with hematoxylin eosin orange, Van Gieson Mallory and Regaud Frozen sections of 4 to 5 micra stained with Sudan III and Bielschowsky

C Microscopic description — a) *Topographic examination* — Microscope Zeiss objective 16 mm , double Mobih ocular N 15

Two months after transplantation union between the graft and the host tissue is complete and formed by

well developed connective and vascular tissue. Topographically, two zones are distinguishable. A peripheral zone, some 2 mm thick, contains epithelial and connective tissue elements, of which a detailed description will be given. A central region consists of necrosed and mummified epithelial and connective tissue, requiring only brief description.

In the living and peripheral region, interpretation requires some skill and familiarity with the histology of grafts Sections examined with but little enlargement show a very heterogeneous texture (Figs. 23 and 24)

However, two different structures show the presence of testicular tissue First, there are small islands of epithelial tissue surrounded by connective tissue. Their diameters range from 50 to 80 micra, and they consist of groups of prismatic cells, 20 to 25 micra across which are heaped together They are clearly outlined and the connective tissue enclosing them is very dense at their borders The second structure consists of groups, each group containing a few tens of small lymphocytiform nuclei, imbedded in a syncytial protoplasmic mass

These two structures, lying 40 to 50 micra apart, often occur together in the same field. The connective tissue consists of closely woven young and adult fibrillar and cellular elements, arranged in concentric layers about the epithelial island groups. The numerous vessels and the infrequency of infiltrated elements are noticeable

b) *Cytologic examination* — With high enlargement, the epithelial tissue presents three forms. 1. There are

Fig 23

Testicular graft removed after 2 months

(Enlargement 60 diameters)

1 Peripheral vasculo-connective tissue region of attachment — 2 Central testicular tissue undergoing necrobiosis — 3 Seminiferous tubule transformed into an epithelial group — 4 Seminiferous tubule transformed into a pseudo-follicle — 5 Interstitial connective tissue. — 6 Interstitial cell — 7 Leucocytic and connective tissue barrier

islands of prismatic cells clearly limited by a connective tissue barrier, as indicated in the topographic examination. 2 There are pseudo-follicular formations, equally well differentiated amid connective tissue 3. Isolated epithelial cells, in small groups, are sparsely distributed amid connective tissue We will examine them separately

1 — Each island consists of 10 to 15 prismatic cells, closely packed together Each cell contains an amblychromatic nucleus, a distinct nucleolus and cytoplasm containing many vacuoles, and granules and sometimes enclosing crystalline structures. Such is the aspect of sections stained with hematoxylin-eosin-orange, and after imbedding in paraffin. In frozen sections fixed in Flemming solution and stained with Sudan, the cytoplasm is seen to contain small unequal granules of neutral fat, coloured red, and small, osmiophilic granules, of lipoid nature Staining by the Regaud method after fixation with hyperchromic Zenker fluid reveals numerous mitochondria, arranged about the nucleus without any distinct polar grouping. The cells are very clearly limited, each being separated from its neighbours by a fine, membranous border.

2 The pseudo-follicular formations are from 120 to 150 micra in diameter They consist of groups of a few tens of lymphocytiform trachichromatic nuclei, of 6 to 7 micra across, buried in a common syncytial cytoplasm Peripherally, there is a very fine reticular tissue, especially well shown by the Bielschowsky staining. Inward,

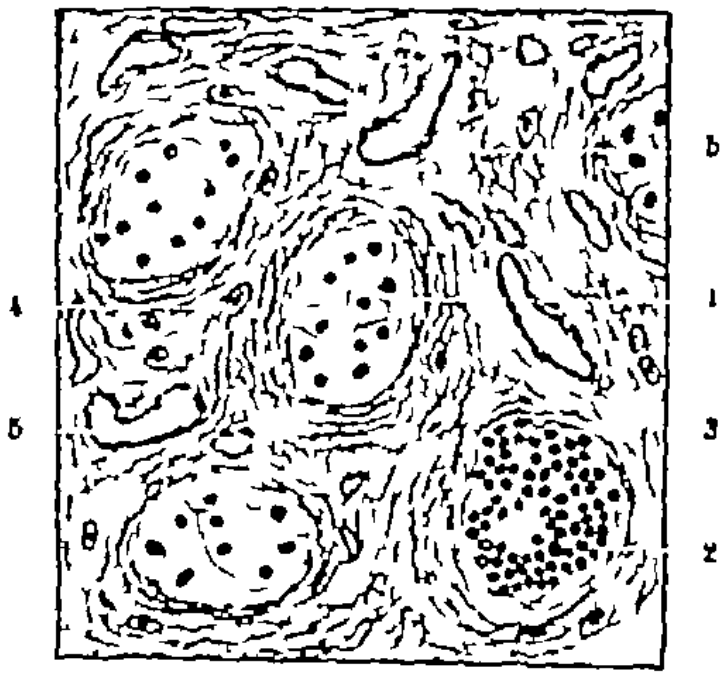

Fig 25

Testicular graft removed after 2 months

(Enlargement 180 diameters)

1 Seminiferous tubule transformed into an epithelial group — 2 Seminiferous tubule transformed into a pseudo-follicle — 3 Interseminiferous connective tissue — 4 Interstitial Leydig cell — 5 Newly formed vessel

this tissue penetrates the first layers of cells Outward, it gradually extends among other interseminiferous connective tissue elements.

3. The epithelial cells, isolated or existing in small groups, are distributed in the connective tissue without order, especially about the insular structures When isolated, these cells are round ; when forming groups of two or three, they are prismatic Their dimensions and structure seem exactly the same as those of the prismatic insular cells, It is needless to discuss them fully here. We shall consider their morphology and significance when speaking of the general histological evolution of testicular grafts

The connective tissue presents nothing special, save its notable development. It presents many connective tissue fibres, a rich network of elastic tissue, collagen, many young fibroblasts, very few infiltrated cells (polynuclears, lymphocytes and red cells) and a relatively large number of plasma cells The blood vessels are remarkably abundant, consisting of capillaries and vessels of small and medium calibre

High enlargement of the region of adhesion shows complete vascular and connective tissue union between the graft and the host tissues It is unnecessary to dwell upon the necrosed central region, in which nearly all the epithelial and connective tissue elements are stained in mass and present only a vague structure, still suggesting the aspect of a testicular section.

Summary · Two months after transplantation, the

graft is completely united with the host tissue by an intimate bond of vasculo connective tissue The general appearance is profoundly altered Peripherally, to a depth of 2 to 2 5 mm , appears the only living tissue present The entire central portion is necrotic In the living portion the epithelial elements of the testicle persist under three forms, namely as islands, as pseudo follicles and as cells scattered throughout the connective tissue The mesenchymatous tissue is highly developed and has the characters of an adult tissue, containing many vessels and a few cells which have infiltrated from without

6 Homoplastic graft, Dog N° 133 Removed after six months (May, 1929)

A Macroscopic description — Graft 6 mm long 4 mm wide and 4 mm thick Colour yellowish white Consistency firm and elastic

B Technique — Fixation with Zenker Helly and formol ammonium bromide Sections of 1 to 3 micra, from pieces imbedded in paraffin and stained with hematoxylin-eosin-orange Von Gieson Mallory and Regaud Frozen sections of 5 micra, stained with Sudan III and Bielschowsky

C Microscopic description — a) *Topographic examination* — Microscope Zeiss, objective 16 mm Mobili ocular N 15

The description of this and the following grafts will be brief because, with slightly varying quantitative relations between the epithelial and connective tissue, the graft structure is very similar for the entire period between two months and four and one-half years after grafting. Our histological studies do not extend beyond the latter period.

This graft, of the six months' period shows complete union with and adhesion to the host tissues (Fig. 25). The epithelial tissue is represented by small islands, clearly limited, and lying amid connective tissue. Their diameter does not exceed 80 micra Each island consists of a group of about ten prismatic cells, of 20 to 25 micra in diameter Each field contains 8 or 10 of these insular formations The second epithelial structures are pseudo-follicles, the field not containing more than three or four of them. In the connective tissue, the fibres clearly predominate, there are relatively few infiltrated cells, and the vessels are evident and constantly present

b) *Cytologic examination* — Microscope Zeiss, 1/12 immersion objective, double Mobili ocular N° 15 enlargement 2 000 diameters.

As with the two-months' graft described above, the high enlargement shows three forms of the epithelial elements, consisting of islands, pseudo-follicles and isolated cells (Figs 26 and 27) Their size, form and structure are identical with those of the similar elements described above and repeated description of them is unnecessary However, the isolated cells are relatively

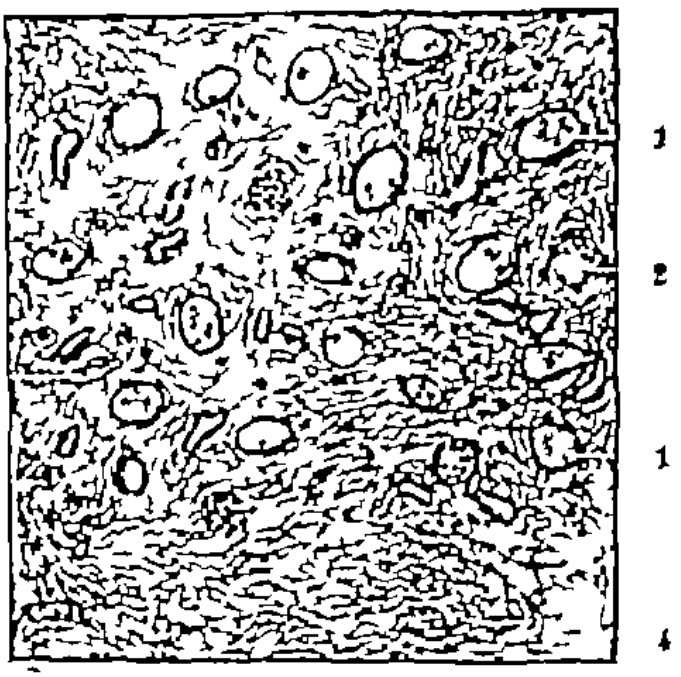

Fig 25

Testicular graft removed after 6 months
(*Enlargement 40 diameters*)

1 Seminiferous tubule transformed into an epithelial island — 2 Seminiferous tubule transformed into a pseudo-follicle — 3 Adult interseminiferous connective tissue — 4 Necrobiotic connective tissue (central zone) — 5 Interstitial Leydig cell — 6 Newly formed vessel

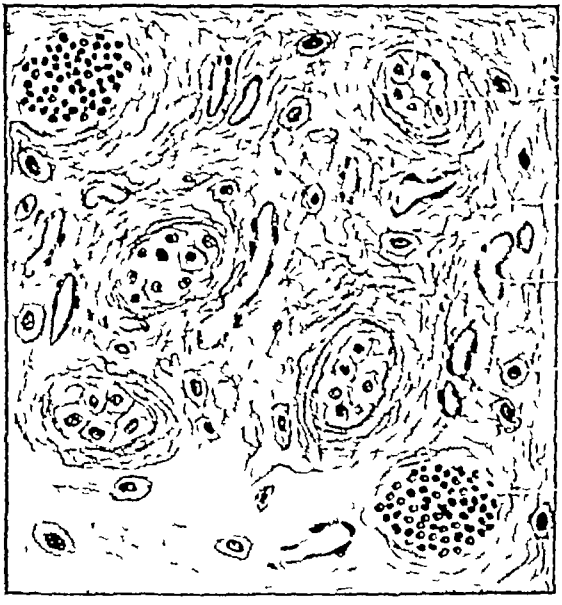

Fig 26

Testicular graft removed after 6 months.

(*Enlargement* 180 *diameters*)

1 Seminiferous tubule transformed into an epithelial island — 2 Seminiferous tubule transformed into a pseudo-follicle — 3 Adult interseminiferous connective tissue — 4 Interstitial Leydig cell — 5 Newly formed vessel

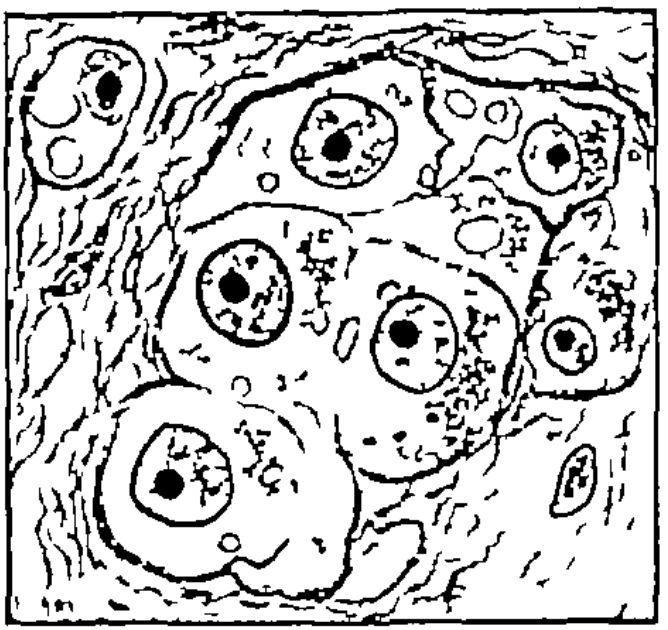

FIG 2

Testicular graft removed after 6 months

(Immersion objective 2.000 diameters)

1 Epithelial group formed of large prismatic cells of glandular appearance (*remains of seminiferous tubules*) — 2 *Large cell of glandular aspect* isolated in the connective tissue — 3 Interstitial connective tissue

much more numerous, while the follicular formations are smaller and less frequent Some of the isolated cells contain mitotic figures

The connective tissue consists of a densely woven fabric of elastic and connective tissue fibres, in whose meshes occur the fixed and mobile elements of ordinary connective tissue. The centre of the graft contains a mass of mummified and necrotic tissue, clearly separated from the living tissue by a lympho-connective tissue barrier

Summary . Six months after transplantation, union between the graft and host tissues is wholly organized, consisting of adult connective tissue and numerous vessels In the centre of the graft, there is a small, mummified portion, surrounded by a lympho-connective tissue barrier The living and peripheral portion of the graft contains epithelial tissue, represented by small islands of glandular, prismatic cells, small pseudo-follicular collections and cells scattered sparsely throughout the mesenchymatous tissue The connective tissue is abundant and contains adult elements and very few cells derived from infiltration. The vessels show all stages of transition from capillaries to vessels of medium calibre

7. Homoplastic graft, Ram N° 15. Removed after 14 months (Retterer, 1919).

Here we utilize the description made by Retterer concerning fragments of a homograft made in the ram, published in the work entitled, "La glande génitale mâle

et les glandes endocrines " p 70, edition published by Doin Paris, in 1921

He finds in the periphery of the graft formations containing large, polyhedric epithelial cells, provided with nuclei These formations are comparable to the insular epithelial formations already described Retterer also describes follicular groupings, 60 to 80 micra in diameter, formed of small and very dense nuclei, 2 to 3 micra in diameter and immersed in a common cytoplasm (Fig 28) Peripherally the small cells of these structures become transformed into young reticular tissue We shall consider this point farther on

The interseminiferous connective tissue has an average thickness of 60 micra between the epithelial structures This connective tissue is adult while young, reticular connective tissue with empty meshes resulting from the transformation of epithelial tissue lies about the follicular formations

Summary As described by Retterer the aspect of the general graft structure after the fourteenth month is on the whole like that described previously The epithelial tissue persists as groups of cells which are morphologically homogeneous in follicles and in epithelial masses while the connective tissue forms a dense and thick fabric

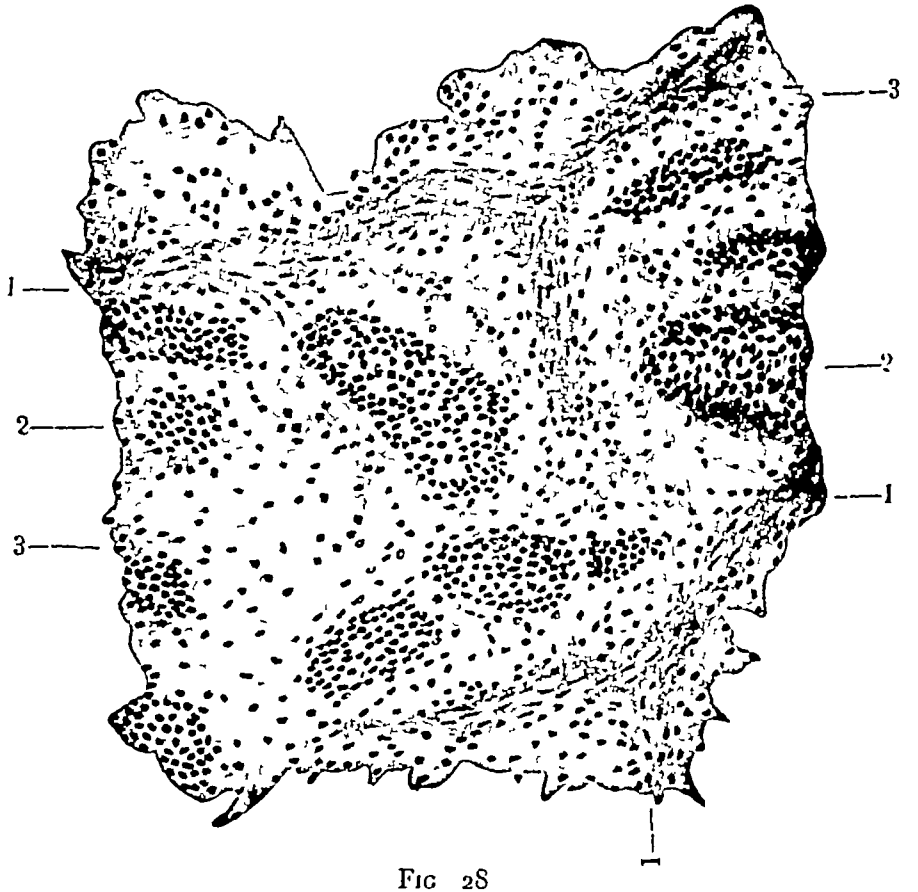

FIG 28

Portion of the grafted testicle of goat No 15, fourteen months after grafting (after Retterer)

Ocular 1, obj 77, Stiassnie — 1 Fibrous traces 2, 2, remains of a seminiferous tubule (closed follicle) 3 3, reticulated tissue, with meshes empty.

8 Homeoplastic graft, between ape and man Removed after two and one half years (S Voronoff, March, 1927)

Here we summarize the histological examination of testicular grafts of the ape transplanted to man and removed after two years and five months This examination was made and described by Retterer in the *Journal d Urologie* tome XXIV No 2 August 1926

The grafts examined numbered three Their dimensions varied from lengths of 6 to 10 mm widths of 3 to 4 mm and thicknesses of 1 5 to 2 mm In one of these graft remains which we summarily describe, the central portion for about one third of the total surface, was necrosed The peripheral portion of two thirds the total surface consisted of living epithelial and connective tissue elements Retterer describes the epithelial formations and gives drawings Some of the formations present columns with central lumen while others are pseudo follicular and suggest the formations present in the fifteen months graft (Figs 29 and 30)

He also describes in the connective tissue stroma of the graft small groups of epithelial cells undergoing transformation into connective tissue In this specimen Retterer finds and stresses anew the persistence of epithelial tissue having the same general arrangement Save concerning points of interpretation to which we shall return the histological description given by Retterer agrees with our own results

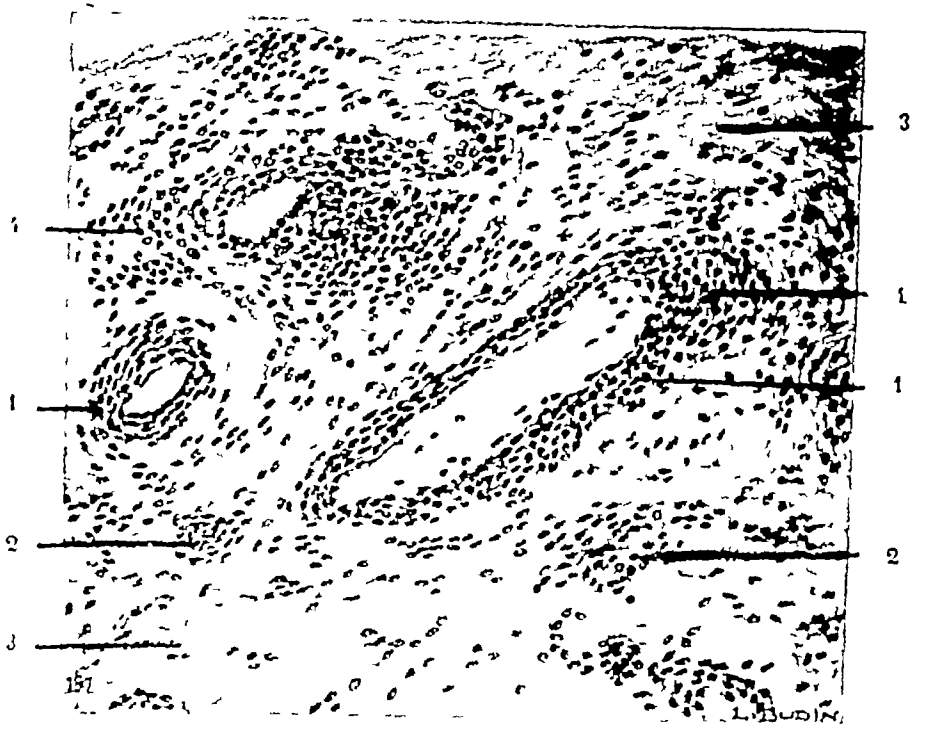

FIG 29

Testicular graft after two and one-half years. Region from Fragment B (after Retterer)

1 Tubules with central lumen, covered with a layer of epithelial cells arranged concentrically whose external portions are being transformed into connective tissue (1) — 2 Masses of full cellular columns in cross-section — 3 Connective tissue stroma

Fig 30

Testicular graft after two and one half years (Retterer) **Small region from the peripheral or cortical portion of Fragment A**

1 Tubules with central lumen covered with a layer of small epithelial cells — 2 Masses of tubules filled with cells being transformed into connective tissue — 3 Connective tissue stroma

Summary In a testicular graft removed two years and a half after implantation in man, epithelial elements occur as full columns, with pseudo-follicles remaining from the seminiferous tubules These elements are surrounded by dense connective tissue, but the follicular structures are always surrounded by young and reticular connective tissue, with empty meshes.

9. Homeoplastic graft, ape to man. Removed three years and two months after implantation (S. Voronoff, July, 1928).

A. Macroscopic description — Graft, 8 mm. long, 3 mm. wide and 3 mm thick Colour, reddish white Consistency, firm and elastic

B Technique — Fixation with Zenker-Helly, formol-ammonium bromide and Flemming solution Sections of 1 to 3 micra from pieces imbedded in paraffin and stained with hematoxylin-eosin-orange, Van Gieson, Mallory, and Heidenhein. Frozen sections of 5 micra, stained with Sudan III, Nile blue and Bielschowsky-Maresch

C. Microscopic description — a) *Topographic examination* — Microscope, Zeiss ,objective 16 mm , Mobili ocular No 15.

No demarcation between the graft and the host tissues, which are intimately unified by adult vasculo-connective tissue A small central portion appears, which is clearly necrotic, shrunken and uniformly

stained, referring equally to epithelial and connective tissue components This point needs no stressing, topographically or cytologically

It is remarkable however, that some tubules, lying near the living tissue, contain, centrally, heads of spermatozoa of normal staining reactions, which indicate their vitality, at least in some degree In the living and peripheral region, the epithelial tissue is represented by insular formations and pseudo-follicular masses which appear numerous Well limited by lamellar connective tissue these formations consist of groups dispersed amid the connective tissue but, more deeply, many of these alterations are tangential, in such cases forming large, irregular epithelial groups 200 to 300 micra in diameter The pseudo-follicular structures are less numerous and most of them are distributed in the superficial parts of the graft The connective tissue is very abundant and dense and contains many vessels, especially at the peripheral portion (Fig 31)

b) *Cytologic examination* — The epithelial elements whether represented by islands or by pseudo follicles, present the cytologic characters already described and needing no further stressing Concerning the isolated cells these are characteristically numerous and many of them present mitosis The inter epithelial connective tissue presents nothing particular except the presence of a few eosinophilic and giant cells in the portion lying between the necrosed and living zones (Fig 32)

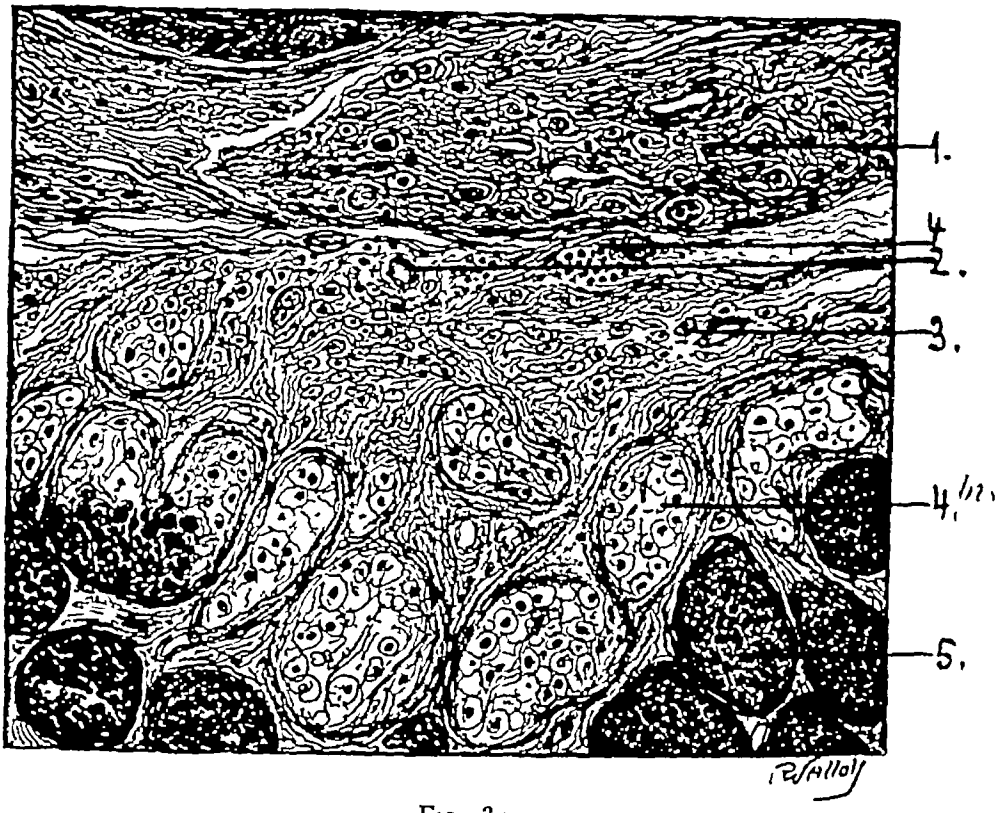

Fig 31

Testicular simian graft, removed after three years and two months

1 Living portion of the graft — 2 Blood vessel — 3 Isolated epithelial cells — 4 Remains of seminiferous tubule (epithelial island) — 4 *bis* Remains of seminiferous tubule (pseudo-follicle) — 5 Mummified seminiferous tubule

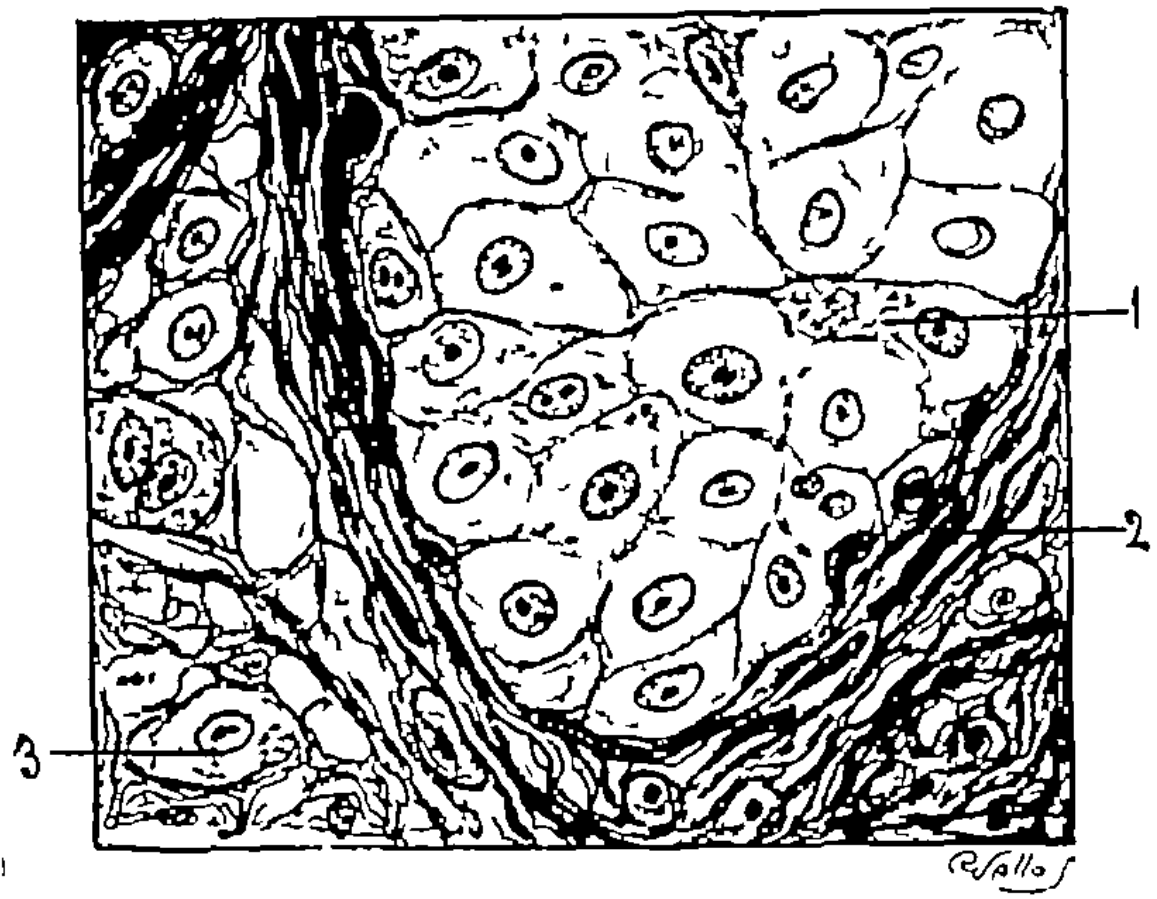

Fig 32

Testicular simian graft removed after three years and two months

1 Glandular cell of an epithelial island — 2 Connective tissue membrane — 3 Isolated epithelial cell

Another feature should be remarked, occurring for the first time in several hundred histological examinations of grafts. This feature consists of the presence of a sensitive Pacini-Vater corpuscle, occurring in the peripheral portion of the graft, in the region of union between the graft and the host tissues. Details concerning the cytologic examination of the necrotic portion are unnecessary A few heads of spermatozoa occur in some of the completely necrobiotic tubules (Fig 33)

Summary : The histological examination of a testicular homeograft made from ape to man, and removed after three years and two months, shows perfect union and undistinguishable differences between graft and host tissues. Vessels penetrate to a depth of about 2 mm at the periphery, the only living part of the graft, This portion contains epithelial elements grouped in islands, together with numerous isolated epithelial cells. Besides the usual fibrillar and cellular elements, the connective tissue contains a few giant and eosinophilic cells and numerous vessels In the zone of union, we find, for the first time, a sensitive Pacini-Vater corpuscle. The central part of the graft is necrosed and mummified It is remarkable to note that a few spermatozoa still persist in some completely necrosed tubules.

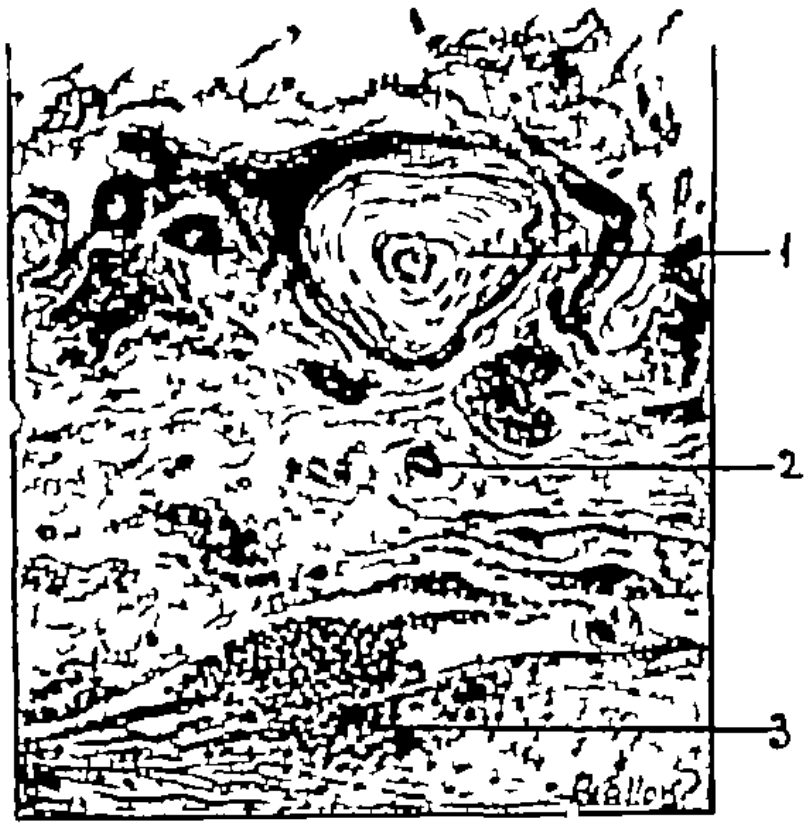

Fig 33

Testicular simian graft removed after three years and two months

1 Sensitive corpuscle — 2 Blood vessel — 3 Lymphofiliform collection

10. Homeoplastic graft, ape to man Removed three and one-half years after implantation (S. Voronoff, November, 1926).

Here we review the histological examination of testicular simian grafts, transplanted to man and removed after three and one-half years. The results of this examination were published by Retterer, in the *Journal d'Urologie*, tome XXIII, No 2, February, 1927.

The remains of this graft consisted of two fragments. One was 10 millimeters long, with an average width of 5 millimeters, while the other was but 3 to 5 millimeters in all its diameters Both were of soft consistency and gelatinous appearance The first consisted only of the so-called mucoid variety of connective tissue The second, and smaller, graft presented, besides the mucoid tissue, portions of more interesting structure

In the second fragment, points consisting wholly of gelatinous, or mucoid, tissue were present, the tubules and epithelial columns being absent Other regions contained the tubules, circumscribed by a membrana propria In still other regions there were full cellular columns, quite surrounded by mucoid tissue.

The points just mentioned have the following structure In a surface having dimensions of about 0 04 mm. (Fig 34), 12 to 15 nodules are present, whose diameters are of 18 micra, 36 micra and, in a few cases, of 50 micra The distance between the nodules ranges from 0,03 to 0 06 mm Some of the nodules represent the

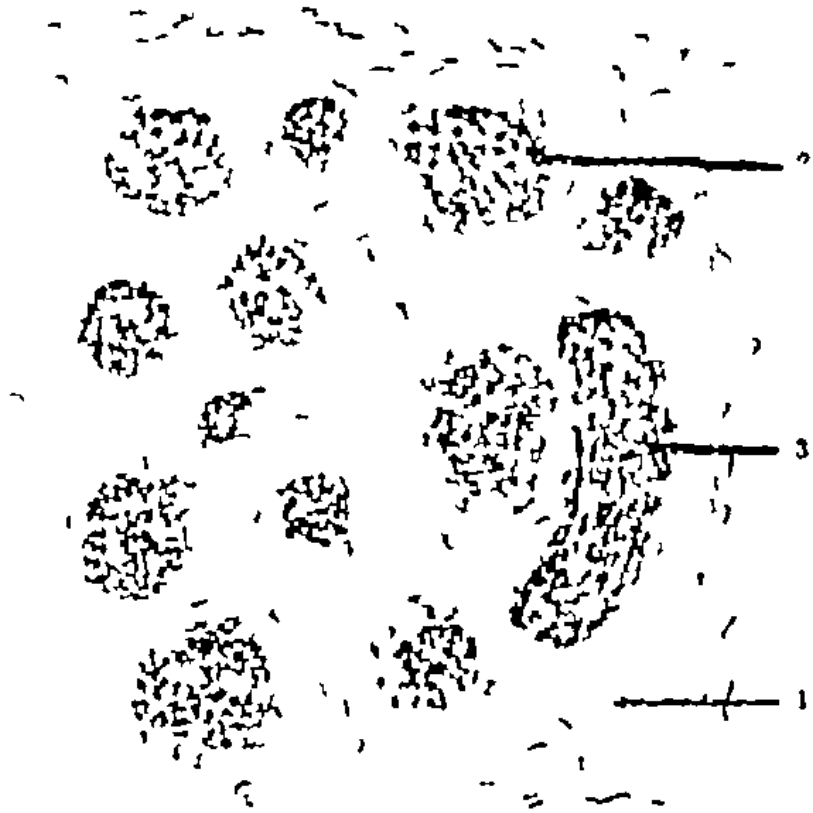

Fig. 34

Cellular columns and intercolumnar substance of the Papion testicle after surviving in man for three and one half years (after Retterer)

1 Intercolumnar tissue — 2 Cross section of column — 3 Longitudinal section of column

remains of cellular columns. The space between the nodules and remains is occupied by gelatinous connective tissue

In regions where the columns exist, the latter present various stages of evolution, the structure differing Figure 35 shows a column, sectioned more or less obliquely and surrounded by the intercolumnar mass (1), which is completely gelatinous. The column has internuclear cytoplasm which is very granular and which stains very strongly with hematoxylin, but the nuclei are surrounded by homogeneous protoplasm, or hyaloplasm (2). At several points there are two nuclei, contained in a single envelope of hyaloplasm

In comparing this appearance with that of epidermis irritated by ammonia or some other vesicant, it is immediately suggested that the epithelial cells of the column present the same stage of formative stimulation as do the epidermal elements Both present perinuclear development of the hyaloplasm and division of the nucleus.

Figure 36 is very instructive, for it indicates the origin of the columns and throws light upon the transformation of epithelial cells into reticular tissue. Nodules (1) are present whose diameters range from 0 01 to 0 04 mm They consist of granular cytoplasm, through which are scattered small and highly chromatic nuclei 3 to 4 micra in diameter At their periphery they insensibly merge into the reticular or mucoid tissue containing them whose extent is about twice as great as that of the nodules and columns taken together We

may at once remark that the nuclei immediately surrounding the nodules and columns are arranged in a layer which is concentric with the columns, near which they are more densely packed and more numerous than in regions more remote Besides the nodules (1) resembling those just described formations exist like those shown at 2 and 3 These structures differ in several ways from the nodules and full cellular columns 1 Their diameters are of 0 06 to 0 08 mm 2 They are separated from the intercolumnar connective tissue by a membrana propria 3 Their contents do not wholly fill the lumen of the tubule Of the latter, some (1) present only a small central mass separated, together with the membrana propria by an empty space or circle To others (2) small masses adhere at one side to the membrana propria Still others (3) are well filled but consist only of granular cytoplasm containing a few small nuclei

Epithelial pearls also occur The contents of seminiferous tubules still supplied with a membrana propria consist of elongated cells which are flattened and lie imbricated together in concentric layers The peripheral epithelial cells of the tubule have disappeared, but similar arrangements namely the little epithelial pearls, exist in many of the columns enclosed in the columnar mass of the granular protoplasm The columns are not simple but ramify as shown by comparison with the structure of the physiological testicle It is a familiar fact that after allowing the testicle to

macerate in an aqueous dilution of nitric acid, anatomists have succeeded in unrolling the seminiferous tubules and have described secondary divisions in relation to those of neighbouring or more or less remote tubules In other words, the seminiferous tubules supposedly anastomose and form a real network, or reticulum I have noticed at several points that an epithelial column may bifurcate to form two branches, whose total diameter is nearly the same as that of the original trunk.

Nowhere, however, have I observed the anastomotic reticulum I am inclined to believe that the appearances described by the anatomists in question are due to the more or less gross procedure constituted by the maceration mentioned, which creates the appearance of anastomosis by failing to destroy certain points of adhesion which meet or cross between two neighbouring tubules In the sections, there occur only the dichotomic divisions like those present in glands, whose ducts ramify in the form of glandular culs-de-sac In the testicle, however, these culs-de-sac are very long and form very marked and numerous turns and loops

After describing the various parts of the graft, a general glance should be cast on the structural elements and appearances just reviewed, in order to show these elements in their proper relations. After three and one-half years following the grafting procedure, the testicular tissues become so changed that colleagues, who are professional histologists, to whom I have shown the

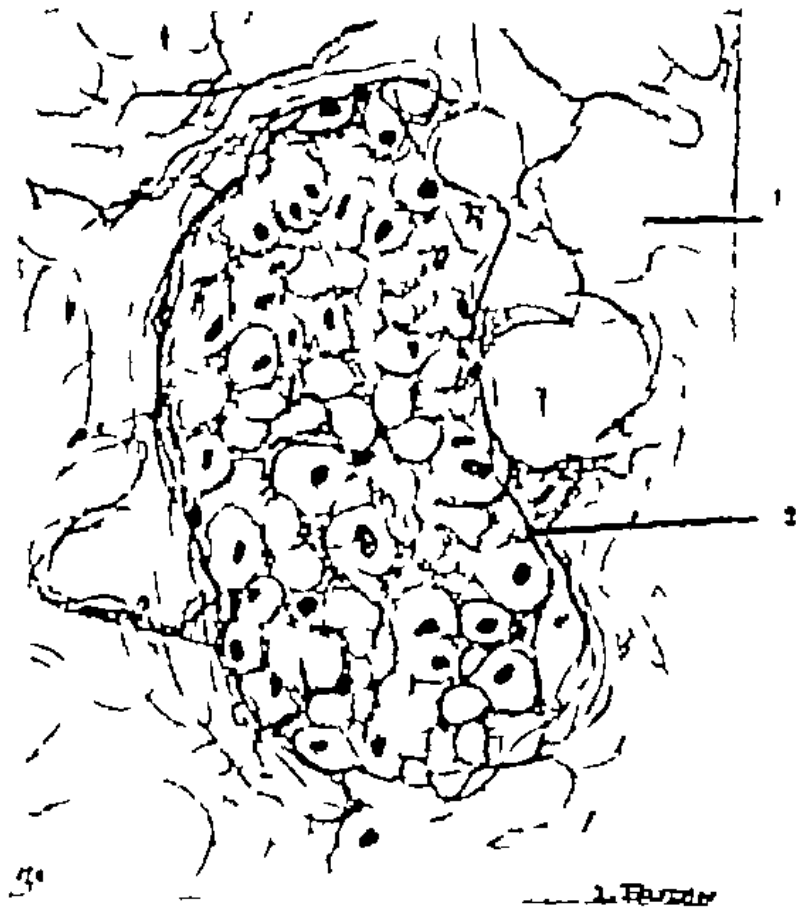

Fig 35

Testicular graft after three and one-half years (after Retterer)

1 Intercolumnar tissue — 2 Nodule

preparations, have been unable to recognize the sectioned tissues. It is a fundamental trait of the grafted testicular tissue that it not only continues to assimilate and catabolize, but undergoes continual alterations. Its form and structure, equally with the accompanying physiological phenomena, are in a state of perpetual transformation. Not only this, but the modifications are not identical in all parts of the graft, probably owing to special sites and more or less favourable conditions of nutrition.

At the moment of grafting, ninety-nine one-hundredths of the graft are composed of epithelial cells having protoplasm which is abundant, clear and finely reticulated. These cells are separated from the connective tissue by a ring of flattened cells forming the membrana propria. The membrana propria persists (Fig. 36) about a certain number of the tubules, in which conditions the epithelium regresses (2 and 3, Fig. 36). The cytoplasm forms a common mass, through which are scattered small nuclei. In other tubules, on the contrary (1, Fig. 36), the membrana propria disappears and the epithelial mass becomes transformed, from the periphery toward the centre, into a series of zones of reticular tissue. The changes undergone by the epithelial cells are identical with those observed in the epithelium of the epidermis when it is exposed to irritation. The nuclei become surrounded by clear cytoplasm, the peripheral cytoplasm becomes granular, and many cells become divided, since the clear cytoplasm of a single

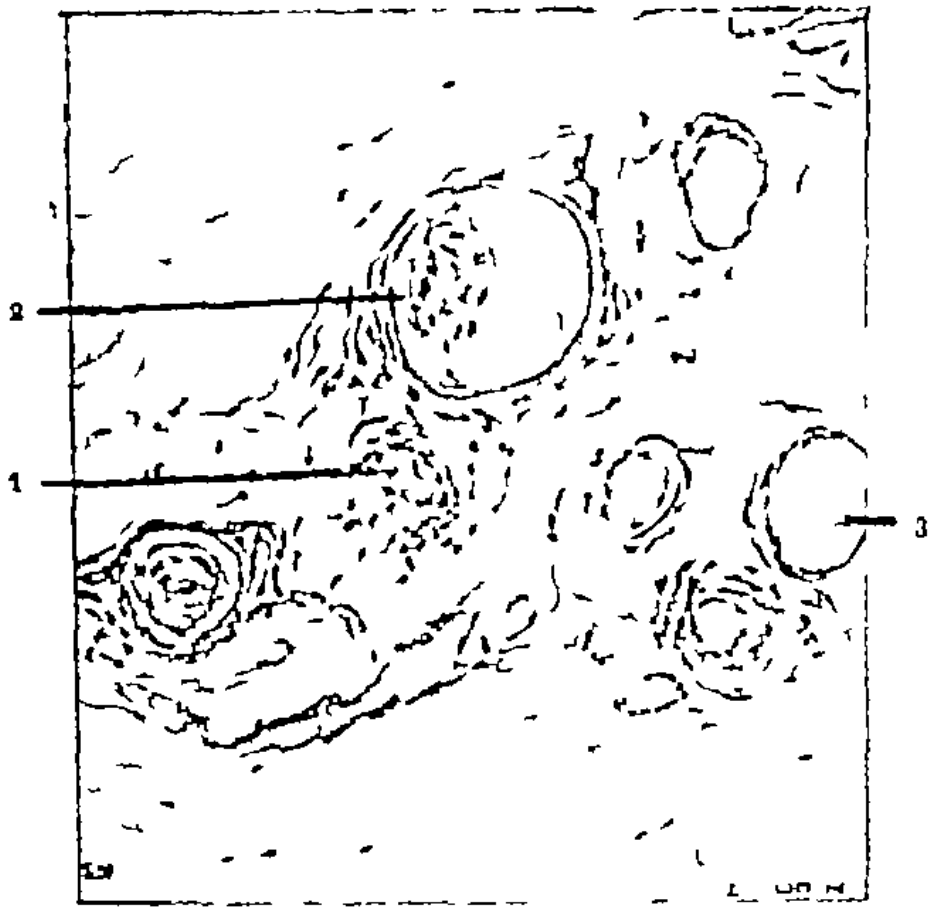

Fig 30

Testicular graft after three and one half years (after Retterer)
Section of a region still containing seminiferous tubules

1 Cellular column — 2 Tubule with epithelial mass and an empty space — 3 Cellular column containing a degenerating mass with a few infrequent nuclei

cell contains two nuclei Both nuclear and cellular divisions occur, and a homogeneous, or young, protoplasm develops. The latter soon evolves like the original cellular cytoplasm, and becomes similarly granular In this way, the epithelial columns become transformed into a cellular complex whose protoplasm is very granular dense and darkly staining The elements of this complex assume a direction parallel to the longer axis of the column.

Summary In a testicular graft removed three and one half years after implantation in man, the histological examination made by Retterer shows the persistence of the epithelial tissue of the testicle, in the form of well-filled columns of cells and epithelial nodules. The elements are surrounded by abundant connective tissue.

11. Homeoplastic graft made from chimpanzee to man. Removed after four and one-half years (S. Voronoff, May, 1928).

Histological examination published by one of us, in collaboration with Retterer, Journal d'Urologie, *tome XXII, No 2, August*, 1928

I Macroscopic description — Four grafts were removed. Their dimensions varied from lengths of 8 to 12 mm., widths of 4 to 6 mm. and thicknesses of 3 to 5 mm The central portions were yellowish

white the peripheral parts reddish white, in colour Consistency firm and elastic Macroscopic sections of the graft remains showed in the larger pieces yellow points of pinhead size containing a pasty homogeneous substance

B Technique — Fixation with Zenker Helly formol ammonium bromide and Flemming solution Sections of 1 to 3 micra from pieces imbedded in paraffin and stained with hematoxylin-eosin orange Van Gieson Mallory and Heidenhein Frozen sections of 5 micra were stained with scharlach

C Microscopic description — a) *Topographic examination* — Microscope Zeiss objective 16 mm Mobil ocular No 15

Of the four grafts removed as indicated above we will describe only the one presenting the largest number of characteristic details designated as fragment A in our work previously published

The central portion recalls the general structure of the testicle but is entirely necrosed Necrosis of the epithelial elements is complete but the connective tissue preserves some of the normal staining and morphological characters showing vitality which is however much reduced The living peripheral portion also presents microscopic points of necrosis encircled by very dense connective tissue Many wholly fibrous points exist formed solely of very dense connective tissue fibres without cells or vessels The remainder of the

surface of the peripheral zone shows the aspect present in the preceding grafts The epithelial islands, 150 micra in diameter, are but few in number, the surface of an entire section containing but four of them. Pseudo-follicular structures and disseminated epithelial cells also exist (Figs 37 and 38)

The connective tissue presents its usual characters, fibrous tissue predominating Elements derived from infiltration are very few, but the vessels are relatively numerous Positively no demarcation exists between the graft and the host tissues

b) *Cytologic examination* — The aspect of the insular formations has become somewhat altered Epithelial collections no longer exist Only crown-like formations of cells, with central lumen, occur The structures are encircled by very dense and abundant connective tissue In general, their aspect resembles that of tubules present in old, cryptorchidic testicles and reduced to the layer of Sertoli cells These intratubular cells are smaller than the prismatic cells previously described. They are 20 to 25 micra in height and 12 to 15 micra in width They are fairly well delimited at the basal region, but the cytoplasm thins out at the apical region and joins a similar prolongation arriving from other cells An irregular cytoplasmic reticulum is thus formed The cells contain few enclosures or granules, and the cytoplasm presents a certain homogeneity (Fig 39).

The basal portion contains a few mitochondrial granules The pseudo-follicular formations and isolated

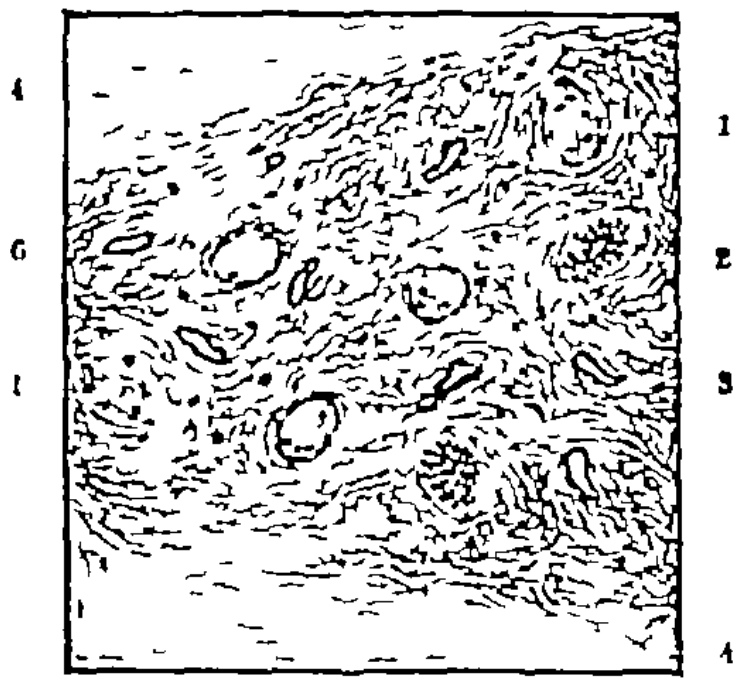

Fig 3-

Testicular graft removed after four and one half years.

(Enlargement 40 diameters)

1 Epithelial island remains of seminiferous tubules — 2 Pseudo-follicle, remains of seminiferous tubules — 3 Fibrous interstitial connective tissue — 4 Necrobiotic tissue — 5 Isolated epithelial cell (Leydig cell) — 6 Blood vessel

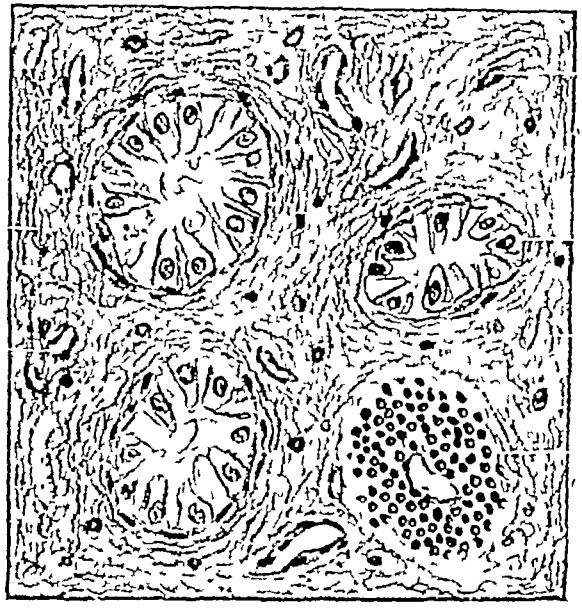

Fig 38

Testicular graft removed after four and one-half years

(Enlargement 180 diameters)

1 Remains of seminiferous tubules lined by cylindrical cells — 2 Pseudo-follicle — 3 Interseminiferous connective tissue — 4 Isolated epithelial cell — 5 Blood vessel

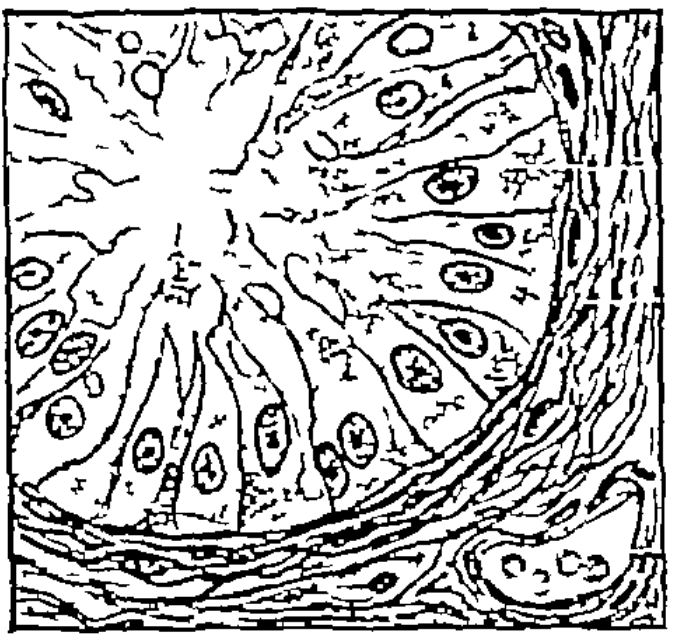

Fig 19

Testicular graft removed after four and one half years.

(Immersion objective 1 100 diameters)

1 Former seminiferous tubule lined with large cylindrical cells of glandular appearance — 2 Connective tissue encircling epithelial elements — 3 Fibrous interstitial connective tissue — 4 Isolated epithelial cell (Leydig cell) — 5 Blood vessel

cells require no comment, save to remark that they are infrequent High enlargement shows that the connective tissue is formed almost wholly of fibres, very few cellular elements existing

Summary · The histological examination of a testicular homeograft, made from chimpanzee to man and removed four and one-half years after implantation, shows complete and undifferentiated union between graft and host tissues The central portion of the graft, as well as a few points in the peripheral region, are mummified, necrosed and encircled by dense connective tissue The epithelial elements are reduced to a few tubules, containing very few cells, and to a few pseudo-follicular formations. But very few isolated epithelial cells exist The connective tissue is well developed and very dense. It is represented almost solely by fibres, only a few elements are present and the vessels are fairly numerous and well developed.

The general histological evolution of testicular grafts

In the preceding portion of the discussion we have shown the histological appearances of testicular grafts removed for examination at different intervals following implantation These intervals mark the more characteristic stages of the morphological transformations which occur From the purely descriptive summary, all critical discussion has been omitted and no bibliographical data have been supplied

Making use of our preceding facts and also utilizing data obtained from many other histological examinations made at intermediate stages of the graft's development we shall endeavour to reconstruct so to speak the general evolution of the grafts from the first few hours to four and one half years after implantation For convenience of description and in consideration of the facts we divide the evolution into four chronological phases which correspond to the concurrent physiological phenomena

First phase continuing from a few hours to four to seven days after implantation

A few hours after implantation the first notable fact appears consisting of imbibition of the grafted tissue by plasma derived from the host with consequent separation of the tubules and disintegration of the intertubular connective tissue Then attracted by positive

chemotaxis, elements from the blood and connective tissue of the host migrate and infiltrate into the peripheral region of the graft, among its seminiferous tubules. Of these infiltrating cells, polynuclears are most numerous, red cells and plasma cells being fewer. During the first two or three days after implantation, the seminiferous tubules remain unaltered, except that their general aspect changes through the multiplication of the cells, which thus fill the tubules completely and transform them into full cellular columns.

Spermatogenesis continues, and spermatozoa fill the central part of the tubules, infiltrating at the same time the rows of spermatocytes and spermatids After the fourth day, the dual infiltration by cells and plasma occupies all the spaces between the cellular columns, both at the periphery and centre of the graft Appearances are as follows at the close of this phase.

Peripherally, the tubules are transformed into full columns of cells which preserve full vitality In the centre, a few tubules show signs of autolysis in the last cellular layers. The interseminiferous tissue consists of young and loosely woven connective tissue infiltrated by many polynuclear, plasma, lymphocytic and hematic cells. Nutrition is accomplished only through imbibition and plasmo-cellular infiltration. Newly formed capillaries rarely appear before the fourth day, almost always appearing between the sixth and eighth days

Second phase, from the end of the first week to the first, second or third month

During this period, the graft undergoes a sort of rearrangement and reconstruction Connective tissue penetrates the graft from the host the result being union between the two tissues Vascular penetration accompanies the extension of the connective tissue Cellular infiltration derived from the host becomes much reduced and elements which have already infiltrated into the graft show, in many cases signs of destruction

During the early weeks the seminiferous tubules undergo important changes with respect to form size and structure Some retain a round or oval form with clear demarcation by the basal membrane Others lose this delimiting membrane and the transformed cellular elements are left free in the connective tissue The tubules, ceasing to retain diameters of 150 to 200 micra rarely reach 120 micra in diameter which usually ranges from 60 to 100 micra Their structure becomes altered at the same time Spermatogenesis usually becomes arrested after the first week or so the new generations reaching only the spermatocyte stage rarely gaining the spermatid phase

The cellular elements tend to become morphologically homogeneous The spermatocytes cease to be formed by about the second month and the original tubules are filled only with prismatic cells of glandular appearance, resembling both the Sertoli and the Leydig cells Besides this transformation and accompanying it cells of seminal type present in certain tubules appear to undergo involution and further differentiation finally arri

ving at the formation af small, lymphocytiform elements, immersed in a syncytial mass suggestive of the small, germinal cells of embryonic seminiferous tubules. In this case, the ultimate result consists of the appearance of pseudo-follicles existing in company with the prismatic epithelial cells, grouped in islands, as already described

Finally, many prismatic cells appear in the graft at the beginning of the second month, being either isolated or grouped in small numbers. The origin of the cells is difficult to ascertain Perhaps they are derived from the reproduction of Leydig cells, for, in a few rare instances, we have observed mitotic figures in the interstitial tissue Again, they may possibly originate from cells of the transformed seminiferous tubules which migrated from their natural site to occupy the neighbouring connective tissue.

The interseminiferous connective tissue freely proliferates and, during the second week, it is represented by a finely woven fabric of young fibroblasts and many collagen fibres, which are infiltrated by a small number of cellular elements derived from the host Later, many of the infiltrated elements show signs of degeneration Toward the close of this phase, the connective tissue becomes adult and well elaborated

Third phase, extending from the second or third month to periods of two or three years or to four and one-half years.

Here the histological characters generally undergo no

profound changes capable of altering the characteristic appearances acquired by the end of the preceding phase At the second or third month a sort of quantitative and qualitative equilibrium becomes established between the epithelial and the connective tissue elements of the graft This equilibrium appears to continue for a long time

During some years the epithelial elements continue to be represented by insular formations composed of large prismatic cells of glandular aspect by pseudo follicular structures by groups of lymphocytiform elements surrounded by a syncytial mass and finally by epithelial cells sparsely scattered throughout the connective tissue

Throughout these phases the interepithelial tissue is represented by adult connective tissue which is rich in fibres and vessels and contains relatively few fixed or migrating cellular elements The features chronologically *differentiating the grafts during this period consist* of reciprocal quantitative differences in the relations existing between the epithelial and connective tissue

During the early months some of the epithelial cells become transformed and others multiply so that they are present in fairly large numbers At the close of the second year however the number of the reproducing cells is much less and becomes considerably reduced after the fourth year During this time the connective tissue evolves in an inverse ratio the final result being almost complete fibrosis of the graft

Fourth phase — During this phase, the graft dies It becomes wholly transformed into fibrous tissue or, at least, the epithelial tissue so far diminishes as to become practically inexistent From this moment onward the tissue ceases to be a physiologically active graft, becoming a mere fibrous nodule.

The complete fibrous transformation of the graft seems to occur at the end of a variable period When the graft "does not take," either through faulty technique, through insufficient nutrition or because of humoral incompatibility, its partial or total necrobiosis may occur by the end of the first week. In this case, the graft will undergo absorption, elimination or fibrous transformation during the early weeks following implantation In normal cases, when the graft "takes", the peripheral portion, in spite of the frequently occurring necrobiosis of the central portion, undergoes fibrous transformation very slowly and complete fibrosis occurs relatively very late. As we have shown, fibrous transformation of the graft may remain incomplete, even after four and a half years

Correlation between physiological phenomena and the histological evolution of the grafts

We have presented a picture of the physiological phenomena observed after testicular grafting and have just described the histological phases of the evolution of the graft. A somewhat closer study of the histo physiological relation and the deductions derived from it remains for examination. For clarity of description, we divide this comment into four parts representing four phases.

First phase. — As we have seen, the implanted grafts undergo violent changes during the first four or five days following operation. They have been detached from their nervous and vascular connections, their anatomical continuity has been interrupted, all their functional characters have been disturbed, they have been placed in another humoral medium and the transplanted tissue has been notably altered.

On the other hand, the receiving organism, having been subjected to the implantation of a foreign tissue upon a very sensitive serous membrane constituted by the tunica vaginalis, surely institutes local and general reactions of defense. In operations upon man, we must also reckon with the psychic factor. Since these elements are known, explanation of the physiological

effects observed during the early days following transplantation is possible, up to a certain point. It will be recalled that we have noted marked psychical and sexual excitation as an evident and active phenomenon, but one which is usually very transitory. We do not think that it is due, essentially or directly, to the graft, that is, we do not believe that it is dependent upon a normal and balanced secretion produced by the transplanted tissue. During the first few days, the graft is not a real one, in the stricter sense of the term, since the testicular tissue thus implanted on another organism is scarcely tolerated. It is adapting itself with more or less difficulty to its new environment, it is seeking to establish vital connections and it is occupied in organizing its new existence. We therefore do not believe that the biological conditions surrounding the graft during the early days are compatible with the existence of a hormonal secretion capable of determining the physiological phenomena observed in the host organism during this period.

There is a wide disparity between the hypo-biotrophy of the graft and the functional activity experienced by the host, and there is every reason for believing that the phenomena which occur are only indirectly due to the graft. Perhaps there is massive absorption of preformed hormones, as thought by Carnot and others, or perhaps the effects are due to a viscero-visceral reflex of sympathetic nature, originating in the irritated serous vaginal membrane and having a terminal reac-

tion in the true testicular tissue appearing as stimulation of the production by the tissue of the hormones proper to it It may also be assumed that there is a local irritation, or even partial destruction of vaso constrictor sympathetic fibres the result being a regional vaso dilatation with consequent increase in the local nutrition occurring for some days in the testicles of the host This hypothesis constitutes the basis of the operations devised by Leriche and Doppler Furthermore the temporary psychic stimulation may possibly be due to suggestion especially since the grafting procedure discussed refers to man, a being remarkably suggestible and imaginative

In conclusion, we must again emphasize the point that the disproportionate effects observed during the first few days following implantation of the graft are only indirectly due to the latter As a logical consequence, the rapid cessation of the effects should not be attributed to exhaustion or destruction of the graft At this moment the transplanted tissue is inert and not yet able to manifest functional activity

Second phase — We have remarked that after the very favourable but very brief initial episode the effects of the graft diminish to the great disappointment of the patients and to the gratification of the detractors of the grafting procedure, who often limit their observation to the initially critical phase For, indeed this phase is a really critical one As we have noted the graft is reorganizing its existence during the second stage of its evo-

lution beginning at the close of the first week and terminating after the second or third month. Blood vessels derived from the host organism are now penetrating into the grafted tissue and undergoing formation there. Seminiferous tubules, having no longer to elaborate generative elements, modify their evolution. The mother cells undergo involution, de-differentiate, and recover their primitive properties. Other elements of the tubules, such as the Sertoli cells, and elements of the interstitial tissue (the cells of Leydig) now begin to secrete in the real, or endocrine, sense At the same time, the connective tissue becomes organized at the expense of the young and newly-formed mesenchymatous cells The transplanted tissue has at last become a simple endocrine gland From the practical point of view, the theories and discussions now more ardently engaged in than ever before concerning the respective rôles of the seminal cells and the interstitial cells, with reference to the secretion of the testicular hormone, are of small importance

Only a very few years ago, there seemed to be no doubt whatever concerning the dogma of the interstitial gland, elaborated by Bouin and Ancel and sustained by Tandler and Gross, Sand, Lipschutz, Steinach, Lichtenstern Godschmidt, Thorek and others However, this seemingly permanent dogma has been disturbed by the researches of Plato, Friedman, Kyrle, Mazetti, Stieve, Kitahara and Cejka Finally, studies made during recent years by Retterer, Voronoff, Champy, Gley, Pezard, Car-

ridrot, Bolognesi, Zawadowski and Orban completely disprove the rôle of the interstitial cells in the production of the testicular hormone and in determining morphologically and physiologically the secondary sexual characters of the male

In collaboration with Retterer one of us has discussed this question in detail in many publications which have appeared from time to time thus far Our latest histological studies of which most are published in the present work serve only to confirm our previous opinion The true germinal cells those of the primary tubules constituting the mother cells of the adult tubules as well as the cells of Sertoli appear to be those having the principal if not the exclusive function of producing the testicular hormone

Returning to the question of parallelism between the physiological phenomena and the histological evolution of the grafted tissue we may conclude that during this second phase of histological reorganization of the graft (accompanied by vascular formation de differentiation of the epithelial elements and reconstruction of the connective tissue) the establishment of the hormonal function is *hardly beginning* and *the effects of the graft* upon the host organism are extremely slight

Third phase — This stage usually begins after the first two or three months We now remark the successive appearance of the various morphological and dynamogenic phenomena enumerated above From this moment onward appear the maximal effects present

for three, four or five years. It may be remembered that the histological structure of the graft during this relatively long period is remarkably characteristic and constant. An equilibrium appears to become established between the epithelial tissue, which is transformed but always present, and the well-formed connective tissue The epithelial elements present characters which are clearly of glandular type, namely, abundant cytoplasm, containing a plentiful distribution of fatty granules, together with lipoids and mitochondria.

The constancy of the epithelial elements is thus accompanied by constancy and maximal development of the physiological effects. However, the equilibrium which we have observed between the epithelial tissue and the connective tissue is established at the expense of the former. The epithelial elements, existing under conditions which are abnormal and more difficult than those occurring in their natural environment, become gradually exhausted, degenerate and finally disappear in the course of four or five years, being then replaced by connective tissue At a given moment, the epithelial tissue is quantitatively and qualitatively "below the effective minimum," an expression which we fully accept, with Pezard Thenceforth, the beneficial effects of the graft cease to exist Disappearance of the epithelial tissue of the graft and transformation of the latter into fibrous tissue are followed by cessation of the physiological phenomena.

Fourth phase — Description of this stage is unneces-

sary It follows the termination of the preceding stage It is characterized by complete fibrous transformation of the graft and final disappearance of the physiological phenomena

The host organism resumes the condition which it presented before transplantation Here, again, there is an evident parallelism Absence of the functional cells brings about the disappearance of the physiological phenomena dependent upon them

General conclusions

The work which we have thus far completed with testicular grafting permits the following conclusions

1 Histo-physiological evolution is identical in testicular homografts and homeografts made in the higher mammals and man

2 During the early days following implantation the transplanted tissue, having lost its anatomical integrity, having been detached from its nervous and vascular connections having been placed in another humoral medium and having suffered deviation of its normal function, undergoes transitory anatomical and physiological disorganization

During this period the physiological phenomena occurring disproportionally in the host organism are due neither directly nor wholly to the transplanted tissue We attribute them to a variety of causes, isolated or combined including massive absorption of preformed hormones absorption of autolytic products derived from the necrobiotic central portion of the graft, perhaps the occurrence of local irritative phenomena constituting viscero-visceral reflexes and in part suggestion

3 During the first two or three months after implantation, the transplanted tissue traverses a phase of retarded vital activity and of organization of its anatomical elements It adheres to the tissues of the host

which supply it with nutritive material through newly formed vessels. It is thus adapting itself to its new humoral environment. Its secretory epithelial elements become differentiated for the performance of a single function, namely, the endocrine function. During this period of adaptation and reorganization, the physiological phenomena are at a minimum.

4. After anatomical reconstruction and the new functional orientation, the structure and function of the graft enter a period of equilibrium which, in favourable cases, continues for four to five, and sometimes to six, years, during which time the accompanying physiological effects remain constantly present.

5. Notwithstanding its adaptation and anatomo-physiological equilibrium, the transplanted tissue reproduces with difficulty and imperfectly resists agencies which may attack and injure the host. The epithelial element is gradually replaced by the connective tissue element. When this transformation brings the epithelial tissue below the effective minimum, the effects of the graft cease to exist. When our grafting technique is strictly applied, this final result occurs after four, five or six years following operation.

6. Complete parallelism exists between the histological évolution of testicular grafts and the sequence of physiological phenomena presented by the host.

Statistical summary showing results obtained by applying simian testicular grafts to man

Our method of grafting endocrine glands of the ape upon the human host dates from 1920 but, until this year we believed that the publication of statistics referring to our operations would be inopportune A method attempting to supply the organism with a fresh source of energy and to prolong its vital resistance should be able to meet the test of time Statistical statements dealing with grafts and grafting have therefore small value unless they supply information concerning the duration of the effects produced by the procedure A ten year waiting period, however should be sufficient for ordinary purposes of testing and control and we think that a statistical summary should now be presented

We are in a position to offer an exact tabulation of the effects of grafting, as applied in a number of rather widely differing cases by one of us and by Doctor Georges Voronoff Our statement cannot include all of our cases and very naturally so since it is not always easy or even possible to remain in close touch with every case during a number of years Many individuals who have submitted themselves to the grafting procedure resided in North or South America the

Indies, Australia, Java, the French and English colonies, various parts of Africa and other more or less remote localities. In such cases, it has not always been possible to keep informed as to their condition of health during the prolonged period necessary for a statistical presentation

We therefore offer statistics covering only 475 cases, concerning which the information at our disposal is sufficiently complete Our statement deals largely with individuals residing in various parts of Europe, whose cases we have been able to follow Since the duration of the effects produced by the graft is the principal factor to which the value of our method is due, we have considered as successful cases only those in which physical and mental improvement has persisted for five to six years, in general, and at least, for three years

Among the cases counted as failures we have included not only those in which results were decidedly negative but also those in which favourable effects failed to continue for more than a few months. We have even numbered among the failures cases in which favourable results have not continued for more than two years.

Such is the spirit in which our statistical survey has been prepared Our statement permits a judgment of the effects produced by grafting, in its various applications It is thus shown in which cases success will be practically certain and also in which cases failure is more likely to follow The summary likewise pre-

sents accurate indications for testicular grafting, either with testicular grafts alone or with an association of grafts of the testicle with those of the thyroid, hypophysis, or both according to the clinical conditions accompanying the given case and to the well known properties and action of the different glands in question It is thus quite clearly shown in what proportion durable success may be expected in cases in which a repetition of grafting is considered after cessation of the effects produced by the primary graft

STATISTICAL SUMMARY

of 475 cases of Simian Testicular grafting upon man, performed

by Doctors SERGE and GEORGES VORONOFF

INDICATIONS FOR TESTICULAR GRAFTING	NUMBER	POSITIVE RESULTS		NEGATIVE RESULTS	PERCENTAGES	
		DURATION				
		5 TO 6 YEARS	3 TO 4 YEARS		FAILURE	SUCCESS
Premature senility.						
35 to 50 years						
Diminution in physical vigour and aptitude for mental application	260	150	67	23	10 %	90%
Impaired genital functions						
Senility.						
50 to 85 years						
General feebleness	64	15	31	17	26 %	74 %
Affections common to the epoch						
Impotence						
Prostatitis						
45 to 70 years						
Glandular hypertrophy	22	—	18	4	18 %	82 %
Obesity.						
40 to 60 years						
Diminution of physical vigour and aptitude for mental applic-	12	6	4	2	16 %	84 %

35 to 55 years Following onanism sexual excesses prolonged abstinence prolonged residence in warm climates. *Testicular and hypophyseal grafts*	28	16	0	3	11 %	89 %
Sexual inversion 30 to 35 years	7	4	—	3	43 %	57 %
Infantilism of the genital organs. 18 to 35 years *Testicular thyroid and hypophyseal grafts made one year apart in the order named*	0	5	—	1	11 %	56 %
Anorchidia 25 to 40 years. Following surgical ablation of tuberculous testicles	0	—	2	7	8 %	22 %
Failure of resection of the vas deferens done outside France 50 to 65 years.	26	8	8	10	50 %	60 %
Grafting repeated 5 to 6 years after the primary grafting 65 to 70 years.	32	23	15	1	9 %	91 %

TABLE OF CONTENTS

TABLE OF FIGURES